8th INTERNATIONAL CEREAL NEMATODES SYMPOSIUM

26–29 September 2022
Abant, Bolu, Türkiye
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Soilborne Nematodes and Fungal Pathogens of Cereals: Advances in Management

Soilborne Nematodes and Fungal Pathogens of Cereals: Advances in Management

Proceedings of

the 8th International Cereal Nematode Symposium

26-29 September 2022,

Abant, Türkiye

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Editors

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Background: The International Cereal Nematodes Symposium (ICNS) is an extension to the International Cereal Cyst Nematode Initiative (ICCNI) that was established in 2006, which was led by the former CIMMYT Soil Pathologist Dr Julie M Nicol along with International colleagues including Dr Roger Rivoal and Dr Eric Grenier (INRA, France), Dr Brian Kerry (Rothamsted, United Kingdom), Dr Maurice Moens (ILVO, Belgium), Prof Halil Eleckcioglu (Adana University, Turkey), Dr Necmettin Bolat (GDAR, Turkey) and Prof Deliang Peng (CAAS, China). It aims to create a critical mass of scientific capacity and skills to deliver sustainable solutions by working at both the practical and theoretical levels. It was initially founded as the International Cereal Cyst Nematode Initiative (ICCNI) and later expanded to include all-important genera of cereal nematodes (CN).

Cereal nematodes including both the Cereal Cyst Nematodes (Heterodera spp.; CCNs) and the Root Lesion Nematodes (Pratylenchus spp.; RLNs) are acknowledged as a global economic problem on wheat production systems in developing as well as in developed countries.

During the last 20 years research has been undertaken by a large network of groups including: 1) the ICWIP (ICARDA CIMMYT Wheat Improvement Program) which relates to wheat improvement research in Central Asia, West Asia and North Africa, 2) CIMMYT and the Turkish Ministry of Agriculture and Forestry in collaboration with ICARDA, and 3) our National Program partners from developing countries and several research institutions in Australia and Europe. The objectives of these groups have been to:

1. **Understand the importance and distribution of CCNs and RLNs,** which are widespread in wheat production systems throughout West Asia, North Africa, parts of Central Asia, Northern India and China.
2. **Investigate potential control options with a major emphasis on host** resistance, through validation and integration of resistant sources (traditional and molecular) into wheat germplasm for global wheat production systems (particularly focused on rainfed or supplementary irrigation systems). Rotation has been partially explored.
3. **Provide training for scientists from developing countries.** The 1st ICCNI was held in Antalya, Türkiye and proceedings can be downloaded from the following website: http://www.spipm.cgiar.org/reports. The 2nd workshop was held in Vienna, Austria and the 3rd workshop was held in Adana, Türkiye. The 4th workshop was held in Beijing, China. The 5th workshop in Ankara Türkiye and the proceeding can be downloaded from the following website: https://www.fao.org/3/i4934e/i4934e.pdf. The 6th Symposium was held in Agadir, Morocco and the 7th Symposium was held in New Delhi, India. Those workshops were very successful in identifying the global status of the problem of cereal nematodes and forming networks and partnerships to continue working on these problems.

Because of the significant interactions between nematodes and soil borne fungal pathogens of cereals in farmers' fields, this nematode Symposium held in Türkiye included a very specialized session on fungal soil borne diseases. At the end of the meeting, the organizing committee met and explored ideas of changing the symposium name into the International Symposium of Cereal Soil Borne Pathogens (1st ISCSBP) to take place every 3 years. This Symposium will bring together researchers tasked with developing solutions to manage intractable soil borne pathogens of cereals for which there are currently limited on farm management solutions.

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Dewey decimal classification:

Cover illustration: Characteristic symptoms of Fusarium crown rot in wheat showing browning of the lower stem and pink fungal growth. Grant Hollaway Agriculture Victoria, Australia.

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This proceedings book is dedicated to

the Global Wheat Program at CIMMYT

Foreword

Small grain cereals account for approximately 20% of calories consumed globally, and about half of all crop area globally. Crops such as wheat, rice, barley, oats, rye and triticale therefore are critical to global food supply.

Organizations such as CIMMYT and ICARDA are dedicated to helping farmers in developing countries to produce more with the same amount of land, without increases to costs of production. We are striving to meet this goal through the genetic gains achieved by our breeding programs, however ending global hunger is another matter. Cereals are important sources of energy, fiber and a wide range of minerals and vitamins and as such are not only a source of calories, but critical for nutrition also. To end global hunger however, according to the Ceres 2030 report[1], an estimated USD$14 billion from funding agencies (double current funding levels) in annual investment would be required in addition to USD $19 billion from Governments in the global south and USD$50 billion from the private sector. The context of events in Ukraine, Syria, Afghanistan and very recently in Pakistan, puts even more pressure on the global food system, coping with rising food prices because of the impacts on supply and supply chains caused by conflicts and natural disasters.

Farmers also need cereal varieties that perform well in the context of a changing climate. This includes response to wide variations in temperature and rainfall as well as resistance to the pests and diseases present in their environments. Meeting the needs of farmers and consumers globally depends on our abilities to continue developing new technologies and applying them, not only in breeding, but in on-farm improvements also. CIMMYT and the Global Wheat Program have been building on the work of Nobel Peace Prize Laureate Dr Norman Borlaug since his work catalyzed the green revolution in South Asia in the 1970s. Today CIMMYT-derived varieties can be found on 60 million hectares worldwide, released by our partners around the world and providing high yielding, nutritious, climate and disease resilient varieties to farmers in Africa, Asia, and Latin America.

Soil borne diseases and in particular, plant-parasitic nematodes, are known to be a major constraint to production of small grain cereals. A recent estimate suggested that global yield losses equivalent to about 10% of global production[2] confirming the importance of a continuing research and breeding focus on mitigating damage.

This workshop will be successful if it continues to build on the progress made in the understanding and control of cereal nematodes. Since the launch of this series of workshops, under CIMMYT leadership since 2009, the community of scientists in attendance continue to dedicate themselves to understanding and mitigating the effects of plant-parasitic nematodes. The improvement of cereal productivity incorporating this knowledge continues to be important for reducing the impact they have on producers and consumers of small grain cereals.

Dr Alison Bentley
Global Wheat Program Director
CIMMYT

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[1] Ceres 2030 Report, Cornell, IFPRI and IISD World without hunger, Berlin October, 2020 https://ecommons.cornell.edu/handle/1813/72799.

[2] Dixon J, Braun HJ, Kosina P, Crouch J (Eds.). 'Wheat Facts and Futures 2009.' (CIMMYT, Mexico) https://www.researchgate.net/publication/46472222_Wheat_Facts_and_Futures_2009/link/0f317536d19f288437000000/download.

Foreword

This 8th International Cereal Nematodes Symposium continues a tradition in providing a forum for researchers to unite and share opportunities to develop improved strategies for the management of plant pathogenic nematodes. In recognition that cereal farmers, in addition to managing nematodes, are also managing soil borne fungal pathogens, this Symposium includes a special session on Soilborne Plant Pathogenic Fungi. Both soil borne fungal and nematode parasites co-exist in the same ecological niche within the cereal field's ecosystem and both attack the root systems, reducing the uptake of nutrients and water, especially under conditions of drought stress. They are both widespread, insidious subterranean constraints for which there are limited genetic and/or chemical control options available making their on-farm control difficult. Furthermore, nematodes and fungi interact. They can have synergistic or antagonistic interactions. Nematodes by direct alteration of plant cells and consequent biochemical changes predispose the host plant to invasion by soil borne pathogens. Root rotting fungi can destroy the healthy host needed by obligate parasites such as cereal nematodes. For a holistic approach, we need to consider the entire biotic community in the soil, and how they affect both fungi and nematodes.

Soil borne pathogens (SBPs) are a major biotic constraint to cereal production worldwide. SBPs include microscopic root-rotting fungi and cereal nematodes, which attack roots and crowns of wheat plants, become problematic in rainfed systems where post-anthesis drought stress is common. The most important pathogens are cereal cyst nematodes, root lesion nematodes, the dryland crown and foot rot complex caused mainly by *Fusarium culmorum* and *F. pseudograminearum*. The lesion nematode *Pratylenchus thornei* has been reported to cause yield losses in wheat up to 38-85% in Australia and 12-37% in Mexico. In southern Australia, grain losses caused by P. *neglectus* ranged from 16-23% and 56-74% in some concomitantly infested areas. The cereal cyst nematodes are *Heterodera species* and the most economically important studied species are *Heterodera avenae*, *H. filipjevi* and *H. latipons*. *H. avenae*, the most damaging species on temperate cereals, is a problem worldwide and is the most studied species while *H. filipjevi* is an economically important nematode on cereals due to its widespread distributions around the world. Yield losses due to *H. avenae* ranged from 15-20% in Pakistan, 40-92% in Saudi Arabia, and 23-50% in Australia. In Türkiye, *H. filipjevi* caused up to 50% crop losses in Central Anatolia Plateau conditions and *H. avenae* up to 24% in the Eastern Mediterranean wheat growing regions.

Fusarium spp. is a globally recognized, economically important group of fungi which includes more than one hundred species causing crown rot, foot rot, root rot and head blight in wheat. Yield losses from crown rot have been recorded up to 35% in the Pacific Northwest of America and 25-58% in Australia, mounting to US$13 million and US$80 million, respectively, due to reduced grain yield and quality.

There are examples internationally where plant pathologists, plant breeders and agronomists have worked collaboratively with a shared objective and successfully developed control of yield limiting soil borne pathogens. An example of this is the development and widespread deployment of CCN resistant cereals in Australia that has led to long term control of this devastating pathogen. Through this symposium scientists will have the opportunity to develop collaborations to further facilitate the development of on-farm strategies for control of these intractable soil borne pathogens.

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On behalf of organizers and editors

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CIMMYT (International Maize and Wheat Improvement Centre) and ICARDA (International Centre for Agricultural Research in Dryland Agriculture) are non-profit International Research Centres with the mandate to improve agriculture in developing countries as part of the Consultative Group of International Agriculture Research (CGIAR). CIMMYT's mandate is to improve the productivity of wheat and maize systems through sustainable management and improvement. The Türkiye office works in West Asia, Central Asia, and North Africa. ICARDA programs are currently coordinated from Lebanon, with technical facilities being coordinated from Jordan. ICARDA works regionally in dryland areas on cereal, legume and animal production systems, once again improving productivity of these through sound management practices. ICWIP (ICARDA-CIMMYT Wheat Improvement Program) is the collective effort of both centres to address food security for cereals in West Asia, Central Asia, and North Africa. CIMMYT and ICARDA are gratefully acknowledged for scientific leadership in research on cereal nematodes, technical input, international capacity building and financial support towards this workshop. For further information see www.cimmyt.org and www.icarda.org.

The Food and Agriculture Organization (FAO) of the United Nations is an intergovernmental organization of 194 member nations plus two associate members and the European Union. FAO has headquarters in Rome, Italy and has staff in over 130 countries. The main goals of the FAO are to eradicate hunger, food insecurity and malnutrition, to eliminate poverty and improve economic and social progress for all, and to foster sustainable management and utilization of natural resources (land, water, air, climate and genetic) for the benefit of present and future generations. FAO has endorsed the Rome Declaration on Nutrition and the Framework of Action, which enshrines the right of everyone to have access to safe, sufficient and nutritious food, and commits governments to prevent malnutrition in all its forms. FAO's support in capacity building throughout the world and particularly for printing this book is recognized. For further information see www.fao.org.

Syngenta is one of the world's leading companies with the goal of improving crop productivity in nearly 100 countries. Through world-class science, global reach and commitment to our customers they help to increase crop productivity, protect the environment and improve health and quality of life. Thanks are given for their support towards capacity building and being key sponsor to this workshop. http://www.syngenta.com

The Republic of Türkiye 's Ministry of Food, Agriculture and Livestock (MFAL) is responsible for food safety, veterinary and phytosanitary policy development, and enforcement activities for food and feed safety, animal health, animal welfare and plant health in Türkiye. MFAL is responsible for coordinating agricultural research and development projects in cooperation with the General Directorate of Agricultural Research and Policy (GDAR). The GDAR is the center of the national agricultural research system in Türkiye, with 49 research stations located to best serve the needs of citizens throughout the Republic. GDAR coordinates research on plant breeding and production, plant protection, animal breeding and husbandry, animal health, fishery and aquaculture, food and feed, postharvest technologies, biodiversity/genetic resources, organic agriculture, bio-safety, and soil and water resource management. Thanks is given to MFAL for hosting and financially supporting this workshop. For further information see www.tagem.gov.tr.

Bolu Abant İzzet Baysal University, one of the noteworthy universities in our country, located in an area surrounded by natural beauties between two metropolis İstanbul and Ankara, was founded on 3 July 1992. The university, which has been developing rapidly since its foundation, has campuses in the city center and three districts of Bolu (Gerede, Mengen, Mudurnu). The main campus of the university, namely İzzet Baysal Campus, is located in Gölköy, which is 8 km from the city center and surrounded by a unique natural beauty. The university comprises 16 faculties, 1 institute, 1 schools, 8 vocational schools and 20 research centers. 1.557 faculty members and 1.181 administrative staff in the university are proud to provide a quality and modern educational setting for about 32.064 students.

Disclaimer

This volume contains papers voluntarily contributed by participants of the 8th International Cereal Nematodes Symposium, 25-29 September 2022, Abant, Türkiye.

Information and views expressed in these papers are entirely those of the authors, whose papers have not been formally peer-reviewed to independently confirm the veracity of the content.

Although the editors have taken care to guide contributors, and have assisted in technical editing, nothing in the papers presented in this volume should be taken as representative of the views or recommendations of the publisher, the editors and/or the sponsors who provided support for this Symposium and/or publication.

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1st International Cereal Cyst Nematode Initiative Workshop
21-23 October 2009, Antalya, Türkiye

3rd International Cereal Cyst Nematode Initiative Workshop
22-23 September 2012, Adana, Türkiye

4th International Cereal Nematodes Initiative Workshop
22-24 August 2013, Beijing, China

5th International Cereal Nematodes Symposium,
12-16 September 2015, Ankara, Türkiye

6th International Cereal Nematodes Symposium,
11-15 September 2017, Agadir, Morocco

7th International Cereal Nematodes Symposium,
3-6 November 2019, New Delhi, INDIA

8th International Cereal Nematode Symposium
26-29 September 2022, Abant, Türkiye

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Session I

An update on the status of the global distribution of cereal nematodes

Root lesion and cyst nematodes affecting cereal production in the western USA

SMILEY R.W

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Summary

Root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and cereal cyst nematodes (*Heterodera avenae* and *H. filipjevi*) are serious pests of small grains in the western USA. It is estimated that root lesion nematodes reduce the economic profit from wheat production by US$51 million annually in three Pacific Northwest (PNW) states. Cereal cyst nematodes are estimated to reduce profits from wheat by another US$3 million annually in the PNW. Many studies have been performed to identify the extent of infestations of species and of the economic losses caused by each species. Studies were also performed to evaluate management strategies such as crop rotation, identification of host crops, efficiency of nematicide seed treatments, selecting and breeding cultivars for resistance and tolerance, influence of tillage, and others. Few economically viable management strategies are currently available in the region and growers continue to endure economic losses.

Introduction

The dominant cereal crop in the western USA is winter wheat produced under rainfed conditions. Other important cereal crops include irrigated winter and spring grains (wheat and barley). Precipitation in the region varies from 200 to 600 mm per year, occurring from autumn to spring. Summers are dry and warm to hot.

The most economically important nematodes affecting small grain cereals (wheat, barley, and oats) in the western USA include two species of root lesion nematode (*Pratylenchus neglectus* and *P. thornei*) and two species of cereal cyst nematode (*Heterodera avenae* and *H. filipjevi*). Other species of *Pratylenchus* and other plant-parasitic genera also occur on cereals throughout the USA but are not well documented for reducing yields of these crops. It should be noted that root lesion and cereal cyst nematodes do not appear to cause serious production problems in the eastern USA, where summer rainfall and a wider diversity of economically viable crops are common. Therefore, most research associating plant damage by root lesion and cereal cyst nematodes on cereals has been reported from the western USA. A summary of previous research is presented in reviews by Smiley (2015, 2016, 2018, 2021, 2022), Smiley et al. (2017), and Smiley and Nicol (2009). Short reports of cereal nematode research in the western USA were also published by Smiley and his associates in two proceedings of this symposium, in 2009 and 2015.

Root lesion nematodes

Of eight species in the genus *Pratylenchus* that are parasitic to wheat, *P. neglectus* and *P. thornei* are the species most often associated with yield loss in wheat in the western USA. The greatest level of crop loss occurs in fields infested by *P. thornei*, but the most land area is infested by *P. neglectus*. Yield reductions up to 50% and 37% have been documented by *P. thornei* and *P. neglectus*, respectively. Economic loss estimates for the three PNW states of Idaho, Oregon, and Washington, as reported in 2009, suggested that *P. thornei* and *P. neglectus* reduced the annual production value of winter wheat by US$14 and $19 million, respectively, and of spring wheat by $3 and $13 million, respectively. The overall loss from these species is therefore conservatively estimated at nearly $51 million annually. Almost no control measures have been put into practice more than a decade after that estimate was published.

Plants that become damaged while growing under drought stress are more likely to suffer yield loss compared to plants having sufficient available water. These *Pratylenchus* species are not strongly restricted by soil type and may attain damaging population levels even in the very driest rainfed wheat-producing regions (250 mm annual precipitation) where wheat is mostly produced as a crop of winter wheat (10 month growing cycle) alternated with a long, crop-free fallow (14 months). Large population densities have been detected throughout the depth of root growth in deep soils. *Pratylenchus* species survive in an inactive, dehydrated state (anhydrobiosis) in roots and soil during dry conditions. Populations decline during fallow periods, but high rates of survival have been reported due to anhydrobiosis and possibly also growth of susceptible weeds during the fallow period between crops.

Pratylenchus neglectus and *P. thornei* often occur as mixtures in the same soil. In view of broad host ranges that vary among these species, identification to the species level is an essential prerequisite for most control strategies. DNA- and protein-based techniques are particularly useful for quickly and accurately differentiating and quantifying *P. neglectus* and *P. thornei*, using a single DNA extract from soil. Biological diversity (pathotypes) among populations has not received adequate attention among specimens collected from wheat or barley in the western USA. However, recently, pathotypic specialization was defined for *P. neglectus* affecting barley in the state of Montana.

Management

Few wheat or barley producers actively attempt to manage root-lesion nematode populations in the USA. Theoretically, the greatest long-term production efficiency will be achieved by planting wheat cultivars that are both resistant and tolerant to the most important *Pratylenchus* species in each field. Dual-species resistance will be needed where both species occur.

Research has shown that it is agronomically possible to integrate crop rotations and planting of tolerant wheat or barley cultivars. Resistance is known in regional barley cultivars but not wheat cultivars. Cultivars carrying resistance and/or tolerance traits are seldom also among those cultivars that yield the highest amount of grain at sites where nematodes do not damage the plants. For economic reasons, growers obviously favor cultivars with the highest yields in agronomic trials, which are not specifically conducted on fields known to be infested with these nematodes. Also, cultivars with resistance to *P. neglectus* are not necessarily resistant to *P. thornei*, and cultivars tolerant to *P. neglectus* are not necessarily tolerant to *P. thornei*. Resistance and tolerance to these species are genetically independent. Very few of the cultivars tested thus far are resistant and/or tolerant to both *Pratylenchus* species, making it important to identify which species is present in each field. Wheat cultivars exhibiting tolerance and some resistance to *P. neglectus* and *P. thornei* have been deployed effectively in Australia but not yet in the USA.

Frequent crops of susceptible crops elevate the nematode populations and increase the level of risk to subsequent intolerant crops. Many cultivars of mustard, canola, lentil and chickpea also increase the population of either *P. neglectus* or *P. thornei*, or of both species. Crops that restrict multiplication of *P. neglectus* and/or *P. thornei* include some cultivars of barley, safflower, triticale, flax, and field pea. These crops can reduce the nematode population and improve the yield potential for subsequent intolerant wheat crops, but these crops are not favored by most farmers because they produce lower economic returns than wheat in the PNW 'wheat belt.' Hosting-ability studies have shown that tolerance is both species- and cultivar-specific, within legumes as well as small grain cereals. It is important to conduct hosting-ability studies using locally available cultivars or breeding lines.

Resistance is the most important strategy for reducing populations of root-lesion nematodes. Resistance to these species is quantitative and controlled by at least several genes. Globally, many sources of resistance to these species have been identified in commercial wheat cultivars, Middle East landrace lines, and in wheat relatives such as species of *Aegilops*. Lines of special interest to agronomists, wheat breeders and farmers exhibit resistance to both *P. neglectus* and *P. thornei*. Also of particular interest are lines that convey high levels of both tolerance and resistance to *P. thornei* or *P. neglectus*. Almost all research on resistance and tolerance to *P. neglectus* and *P. thornei* has been performed in Australian and CIMMYT wheat breeding programs. Limited research has been performed in the USA, as summarized in the review papers cited earlier. An important exception is the development of the recombinant inbred wheat line, LouAu, that enabled identification of quantitative trait loci associated with resistance to *P. neglectus* and *P. thornei*. The LouAu population also identified partial resistance to *Fusarium culmorum* and *Rhizoctonia solani* AG8. Six major and 16 speculative quantitative trait loci were identified across seven measured traits. Four of the six major quantitative trait loci were found within the same genomic region of chromosome 5A, suggesting shared gene(s) contributed to the resistance. These quantitative trait loci are useful in breeding programs looking to incorporate resistance to soil-borne pathogens in wheat cultivars.

Other management practices are less effective in managing *Pratylenchus* populations. Field sanitation during the fallow phase is as important as during the in-crop phase, because *Pratylenchus* species have been shown to multiply on many weed species in the genera *Avena*, *Brassica*, *Bromus*, *Carrichtera*, *Emex*, *Heliotropium*, *Hordeum*, *Malva*, *Raphanus*, *Rumex*, and *Tribulis*, and on volunteer cereals. The presence of susceptible weeds or crop species between planted crops allows *Pratylenchus* to increase in population density over a greater time interval of the most prevalent cropping system in the PNW, the 2-year winter wheat-fallow.

Tillage has reportedly increased, decreased, or had no effect on populations of *Pratylenchus* in wheat. Most interpretations of tillage effects on *Pratylenchus* in the USA were based upon soil samples collected at shallow depth. Peak population densities of these nematodes vary from near the surface to as deep as 60 to 90 cm, with significant densities to a depth of 150 cm in some soils. Therefore, shallow samples (to 20 cm depth) used in most surveys and agronomic research trials are now considered inadequate for accurately characterizing *Pratylenchus* populations in many deep soils. Reported effects of tillage on these populations needs to be investigated further.

Pratylenchus neglectus and *P. thornei* are often more damaging to crops in drier than wetter regions. The economic threshold for damage is expected to be lower for rainfed, low-precipitation environments than for crops produced with supplemental irrigation or in areas of greater precipitation during the growing season.

Several chemical nematicides are effective and have been widely used in research but are not registered for commercial use due to economic and environmental concerns. Several biological agents have been effective in carefully controlled studies where plant roots were confined within small greenhouse pots. Thus far, those agents have been ineffective in field trials. None of these agents are currently available for application to vast areas of wheat production area known to be infested by *Pratylenchus* species.

Green-manure crops can be used to sanitize soil through bio-fumigation in regions where water is not a limiting factor for wheat growth, such as in higher precipitation regions or where supplemental irrigation is available. Several bio-fumigant crops, such as Sudan grass and some *Brassica* species, enable *Pratylenchus* species to multiply. When these crops are incorporated as green tissue into soil, toxic products can reduce the elevated population to pre-plant levels or below. But populations elevated on seedling 'biofumigant' plants may remain high where these crops are grown to maturity for harvesting seed or forage, as is practice throughout much of the rainfed wheat production region in the western USA.

Cereal cyst nematodes

Cyst nematodes affecting cereals are placed in the genus *Heterodera* and more specifically into the '*H. avenae* group,' which consists of a complex of 12 species and intraspecific pathotypes that invade roots of cereals and grasses. Of the three species of primary economic importance globally, only *H. avenae* and *H. filipjevi* have been detected on cereals in the USA. Only one of these species is generally identified in most fields but mixtures of species also occur. Each species can cause whole-field losses up to 10% in wheat and barley, but production crops have been destroyed in a small number of fields. Economic loss estimates for the three PNW states of Idaho, Oregon, and Washington, in 2009, suggested that these species reduce the annual production value of wheat by more than US$3 million. Very few control measures have been put into practice since that estimate was revealed.

The presence of *H. avenae* in the three PNW states were first reported during the 1970s. This species is now reported as occurring in at least seven states. A previous lack of recognition is thought to have been more responsible than recent spread for the greater number of detections during recent years. Identification of cereal cyst nematodes is complex and has traditionally been based on comparative morphology and diagnostic keys. It wasn't until the development of a DNA-based diagnostic procedure, in the early 2000s, that it became known that some populations previously reported as '*H. avenae*' are actually *H. filipjevi*. The more precise identification using molecular- and/or protein-based procedures enabled the differentiation of these two species.

"The International Cereal Test Assortment for Defining Cereal Cyst Nematode Pathotypes", first described in 1982, underestimates the polymorphism now known to exist for *H. avenae* populations globally. In the three states of the PNW, the assortment was unable to define virulence groups (pathotypes) of the populations. However, it did establish that the *Cre1* gene for resistance was effective for suppressing or preventing reproduction of all *H. avenae* populations. Less effective but possibly important *Cre* genes for use in gene stacking were also identified. There is still no published report of the pathotype(s) of *H. filipjevi* in the USA but current research toward that objective is occurring in Washington.

Cereal cyst nematode is not strongly restricted by soil type, but damage is often greatest in light-textured, well-drained soils such as sands. The damage threshold varies with soil type, climate, and cultivar, and with nematode species, virulence, and ecotype characteristics. These variable influences on plant damage make it difficult to directly relate initial populations with reduction in grain yield.

Management

To achieve effective control of cereal cyst nematodes it is necessary to reduce the population below the economic threshold for damage. This requires definitive studies on population dynamics and yield losses on representative local cultivars under natural field conditions. Cultural practices based on rotational combinations of non-hosts (non-cereals), resistant cultivars, and clean fallow can effectively control these nematodes. Restricting hosts to 50% of the time in heavier soils and 25% of the time in lighter soils can cause dramatic reductions in the population of *H. avenae*. However, these management strategies each require a full understanding of the virulence and diapause characteristics for the local nematode populations, and of the effectiveness and durability of the resistance gene(s) deployed against that nematode population.

The use of plant host resistance is one of the most effective methods to control cereal nematodes. Resistance is defined as the ability of the host to inhibit nematode multiplication. Ideally resistance should be combined with tolerance, which is the ability of the host plant to maintain yield potential in the presence of the nematode. The use of cultivars that are both resistant and tolerant offers the best control option, in addition to being environmentally sustainable and requiring no additional equipment or cost. As stated earlier, the *Cre1* gene has been shown to be highly effective against populations of *H. avenae* in the USA. However, resistant cultivars will only be used by farmers if the resistant cultivar also maintains a level of yield, in non-infested soils, which is comparable to other commonly cultivated wheat cultivars.

Practices other than crop rotation and planting resistant cultivars are less efficient. Nevertheless, components of those strategies are important for integrated management approaches to nematode control. These components may include manipulating the sowing time to minimize the impact of the major hatching period, as when winter wheat is planted during the autumn in the cool, temperate regions where the major hatch of second-stage juveniles occurs during the spring. Winter wheat if far more mature than newly emerging spring wheat or spring barley during the primary period for hatching of infective *Heterodera* juveniles.

The greatest crop loss occurs when nutrients or water become limiting for maximum plant growth potential at any point during the growing season. Crop damage is therefore minimized by supplying optimal plant nutrition and, where possible, supplemental water during intervals of drought.

A low rate of nematicide application can provide effective control of cereal cyst nematode in wheat. However, environmental concerns associated with these chemicals eliminate them as a viable alternative for rainfed wheat production. Nematicides are potentially economically viable in irrigated crops where wheat or barley are rotated with higher value crops such as potato or sugar beet. At present, nematicides are primarily used as a research tool for studying yield loss and population dynamics.

Current research and additional needs

Surveys have revealed areas where cereal cyst and root-lesion nematodes are present. More intensive surveys are required to define the distribution of species and identity of pathotypes. Experiments are urgently needed to define the magnitude, frequency, and value of yield reductions. Further adoption of existing molecular identification and quantification techniques by commercial soil diagnostic laboratories will facilitate more rapid and effective surveys of these nematode populations. Several commercial labs now provide such diagnostic services but demand for that service appears to be low among growers and advisory personnel.

Few wheat and barley improvement programs are breeding for or selecting lines for resistance or tolerance to these nematodes. For cereal cyst nematode, public and private breeding programs in Idaho and Washington are screening wheat cultivars for resistance and/or tolerance to *H. avenae* or *H. filipjevi*. Studies in Washington are also seeking to identify the pathotype of the local population of *H. filipjevi*. The author of this paper is not aware of any current screenings to identify PNW cultivars of wheat or barley for higher levels of resistance or tolerance to *P. neglectus* or *P. thornei*. It appears likely that this low level of current research is based upon a relatively low level of commercial acceptance of the importance of these nematodes, which results in limited financial, technical, and institutional support for research on nematodes that affect small grain cereals. This research is relatively difficult and labor intensive and requires extensive searching to identify fields with adequate uniformity of nematode infestation as well as low levels of damage caused by soilborne fungal pathogens (species of *Fusarium*, *Pythium* and *Rhizoctonia*) and other plant-parasitic nematodes. These challenges appear to reduce interest among those who need to conduct this field research.

Many years of multi-location field experiments or utilization of rain-out shelters are required to accurately associate field populations with yield loss data. Without such knowledge, it is very difficult to develop management guidelines that associate levels of disease risk with soils, cultivars, climates, and fluctuating weather.

Greater collaboration is needed between research institutions in the USA and international organizations such as CIMMYT and ICARDA, as well as between public and private wheat breeding programs. These collaborative efforts will provide a greater understanding of the complexity, economic importance, and control of *Heterodera* and *Pratylenchus* populations in the western USA. Results of all research findings also need to be more effectively communicated to farmers and their agronomic advisors.

Smiley (2021) reviewed the status and the future role of root lesion nematodes in dryland wheat production systems as the climate changes in the PNW. He summarized by stating that global interest has accelerated for identifying reasons for differences between actual and potentially attainable crop yields. The so-called ‘yield gap’ in the PNW is far greater for rainfed than irrigated wheat, and the gap has remained static as actual yields have increased. Nearly all yield-gap studies continue to focus on water-use efficiency as the most limiting factor restricting crops from reaching their full yield potential given the total amount of water available. Recognition is not being given to impacts of two chronic root and crown diseases (root lesion nematodes and Fusarium crown rot), each of which greatly suppress the ability of plants to extract water from deep within the soil profile. Smiley concluded that the yield gap for rainfed wheat in the PNW cannot be effectively reduced without addressing the roles of these diseases on water-use efficiency.

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Incidence of cereal nematodes in the cereal cropping system in CWANA Regions: Azerbaijan, Kazakhstan, and Kyrgyzstan

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Summary

A nematode survey conducted between 2018-2020 in Azerbaijan, Kazakhstan, and Kyrgyzstan, representing Central and West Asia, revealed that root-lesion and cyst nematodes are widely distributed in several wheat growing regions of the country. Plant parasitic nematodes (PPNs) were detected in 90% of soil samples, eleven genera were identified including *Pratylenchus, Heterodera, Geocenamus, Ditylenchus, Helicotylenchus* or *Rotylenchus, Paratylenchus, Pratylenchoides* and *Tylenchorhynchus*. The root lesion nematodes: *Pratylenchus neglectus* and *P. thornei,* and cyst nematodes; *H. filipjevi* identified by morphological with molecular data. The only *Heterodera* species identified was *H. filipjevi* and it was found in 74 of 233 samples. High infestation rate was recorded from Kazakhstan when compared to other countries; Kyrgyzstan (31%) and Azerbaijan (28%). Intraspecific polymorphism was observed within *H. filipjevi* populations and divided into at least two groups. Two root-lesion species identified were *Pratylenchus neglectus* and *P. thornei* and they found in 52 and 64 of 233 samples, respectively. The cyst nematode populations were identified as *H. filipjevi* occurring separately or in mixed populations with the root-lesion species. The current study was conducted to evaluate the incidence of PPNs in the main cereal growing areas in northern Azerbaijan, Kazakhstan, and Kyrgyzstan.

Introduction

Wheat (*Triticum aestivum*, L.) is the most important food crop of many countries, contributing nearly one-third of the total food grain production in the world (FAOSTAT 2018). One of the main reasons for the low yield potential may be due to the plant parasitic nematodes (PPNs) that are considered one of the most relevant constrains limiting cereal production globally (Dababat and Hendrika 2018). Genera such as *Heterodera* (cyst), *Meloidogyne* (root-knot), *Tylenchorhynchus* and *Merlinius* (stunt), *Ditylenchus* (stem), *Anguina* (seed-gall), *Pratylenchus* (root-lesion), and *Paratrichodorus* (stubby-root) can cause significant yield losses (Rivoal and Cook 1993). The cereal cyst nematodes (CCN) are the most widely studied on wheat and have been reported by many researchers worldwide (Subbotin et al. 2010).

Root-lesion nematodes (*Pratylenchus* spp.) are widespread and one of the most important groups of plant-parasitic nematodes due to their wide host ranges and their worldwide distribution (Jones et al. 2013). According to Castillo and Vovlas (2007) and De Luca et al. (2011), the genus comprises 75 nominal species

(Zamora-Araya et al. 2016), with *Pratylenchus thornei* and *P. neglectus* being two of the most frequently reported species (Yan et al. 2008; Thompson et al. 2016). The cyst nematode, *Heterodera* spp. is important obligate parasites and an economically relevant group of harmful plant parasitic nematodes which are found throughout the world (Subbotin et al. 2010 a, b). The main species parasitizing cultivated wheat are *Heterodera avenae*, *H. filipjevi*, *H. hordecalis* and *H. latipons* (Subbotin et al. 2010). *Heterodera filipjevi* is the most damaging pest that limits cereals production in the major cereal-producing regions of the world including the Central and West Asia (McDonald and Nicol 2005; Dababat and Fourie 2018).

Accurate identification of phytoparasitic nematodes to species level is needed as an initial step in effective control measures. Economically important cereal nematodes: *Pratylenchus* spp. and *Heterodera* spp. very highly morphologically and morphometrically similar to each other. Species identification based on the shape and measurement of second-stage juvenile, female, and male species becomes more complex as the number of species within these genera increases (Subbotin et al. 2003; Handoo 2002). However, molecular techniques make it easier to distinguish closely related species in these genera (Subbotin et al. 2003; Castillo and Vovlas 2007). The D2-D3 expansion segment of 28S gene and the internal transcribed spacer (ITS) show a high polymorphism among the species of these genera; therefore, these regions are a useful tool for species identification and phylogenetic analysis of these cereal nematodes. Also, sequencing the 28S gene and ITS-rDNA of undescribed nematodes species, which published or reserved in GenBank (Tanha Maafi et al. 2003), facilitates the accurate identification for most of the root-lesion and cyst nematode species.

In CWANA region countries particularly: Azerbaijan, Kazakhstan, and Kyrgyzstan, there is also little information on the morphological and molecular characteristics of these root-lesion and cyst nematodes. The aims of the current study were to (i) investigate the root lesion and cyst nematodes densities in the major wheat-growing areas of Azerbaijan, Kazakhstan, and Kyrgyzstan provinces, representing CWANA region (ii) describe the root lesion and cyst nematodes species by morphological and molecular techniques and (iii) evaluate possible intraspecific variation within root lesion and cyst nematodes populations based on the 28S gene and ITS-rDNA regions, respectively.

Methods

Over the three-year period from 2018 to 2020, a total of 233 samples were collected from Azerbaijan, Kazakhstan, and Kyrgyzstan. Prior to the start of sample collection, a systematic sampling protocol was established with sampling by district and parts within the district (no more than one sample per section) subject to road access. Field selection for sampling was based on the criteria that the field was representative of wheat production in that country or nearby region and that field locations were separated by at least 1.6 km (6 to 9 km in most cases). A minimum of 10 cores were taken per sample, then soil was mixed, and a representative of 2 kg subsamples was kept for analyses. Each sample was labelled with sample number, province, coordination, and cultivated crop.

Migratory nematodes were extracted from 100 cm^3 of soil by using a modified “Petri-dish” Baermann funnel method (Hooper 1986). Extracted nematodes were counted at 100x magnification under the light microscope and identified at genus level. Cyst nematodes were extracted from 250 cm^3 of soil by using Fenwick can (Fenwick 1940). At least 10 full cysts were selected and handpicked from each sample and stored at 4 °C to be used in the molecular and morphological analysis. Cysts were morphologically identified to genus level under a stereo-binocular microscope (Zeiss, Jena, Germany, and V20). The incidence of the nematode [(number of samples with nematode / total number of samples) *100] were calculated for each field.

The nematodes were killed in hot water bath and fixed in 2 percent formaldehyde. Nematodes were processed for dehydration by Seinhorst's method and mounted on glass slides in anhydrous glycerin (Seinhorst 1959). Identification of nematode genera and species were based on the morphology with the help of keys (Handoo 2002). The exact number of nematodes in each genus was assessed. The root lesion and cyst nematodes by using morphological traits and molecular markers (ITS, 28 S and 18 S rDNA gene sequences).

Results & discussion

The incidence of PPN populations per 100 g of soil associated with cereal crops at 233 sites from Azerbaijan, Kazakhstan, and Kyrgyzstan was calculated. Plant parasitic nematodes was present in 90% of samples, with an average incidence of 960 individuals/ 100 g soil. Eleven genera of PPNs were identified (Table 2). *Geocenamus* spp. were most prevalent (65%), followed by *Trophurus* spp. (62.5%) and *Pratylenchus* spp. (52.5%). The lowest frequency was recorded for *Pratylenchoides* spp. (20%). Economically important PPNs found in soil cores were *Pratylenchus* and *Heterodera*. (Table 1).

Table 1. Incidence of plant parasitic nematodes associated with wheat in Azerbaijan, Kazakhstan, and Kyrgyzstan region

Genus of Nematode	Incidence (%)	Average
Geocenamus	72	900 ± 60 (200 - 1400)
Trophurus	72	490 ± 20 (400 - 660)
Pratylenchus	64	540 ± 60 (300 - 880)
Ditylenchus	48	580 ± 60 (300 -840)
Helicotylenchus	44	490 ± 50 (380 - 680)
Tylenchus	36	580 ± 30 (420 - 780)
Amplimerlinus	34	640 ± 50 (200 - 980)
Merlinus	46	550 ± 30 (250 - 860)
Paratylenchus	28	680 ± 40 (400 - 920)
Tylenchorhynchus	22	520 ± 30 (140 - 860)
Rotylenchus	20	340 ± 40 (340 - 860)
Pratylenchoides	26	380 ± 30 (120 - 460)

Heterodera species were found in 30% of the cereal fields. Based on the morphological characteristics and molecular analysis, *H. filipjevi* was the only species identified. It was found almost in all fields where monoculture system is practiced (Table 2). The fields where cyst nematodes were not detected were generally rotated with other crops. High cyst incidence levels were recorded, *e.g.* 29 of 82 fields were infested in Kazakhstan. The lowest incidence of infested fields was recorded from Azerbaijan where only 21 out of 74 fields were infested. (Table 2).

Table 2. Incidence of cyst nematodes associated with wheat in Azerbaijan, Kazakhstan, and Kyrgyzstan region

Country	Number of Surveyed areas	Number of infested fields	Incidence (%)	Infestation level (average number of cysts)
Azerbaijan	74	21	28	30.4
Kyrgyzstan	77	24	31	26
Kazakhstan	82	29	35	23.2
Total	233	74	31	26.5

Pratylenchus neglectus and *P. thornei* were found in 22% and 27% of the cereal fields, respectively. Based on the morphological characteristics and molecular analysis, *P. neglectus* and *P. thornei* was the two root lesion nematodes species identified. It was found in almost all the fields where monoculture system is practiced (Table 3). The highest *P. neglectus* incidence levels were recorded, *e.g.* 23 of 82 fields in Kazakhstan. The lowest *P. neglectus* incidence of infested fields was recorded from Kyrgyzstan where only 14 out of 77 fields were infested. (Table 3).

Table 3. Incidence of root lesion nematodes associated with wheat in Azerbaijan, Kazakhstan, and Kyrgyzstan region

Country	Number of Surveyed areas	***Pratylenchus neglectus***		***Pratylenchus thornei***	
		Number of infested fields	**Infestation (%)**	**Number of infested fields**	**Infestation (%)**
Azerbaijan	74	15	20	16	21
Kyrgyzstan	77	14	18	22	23
Kazakhstan	82	23	28	26	34
Total	233	52	22	64	27

This survey provides information on occurrence and density of plant parasitic nematodes associated with wheat and barley crops cultivated in Azerbaijan, Kazakhstan, and Kyrgyzstan. The results of this study indicate that some plant parasitic nematodes especially the cereal cyst, and root lesion nematodes, the endoparasites, were found widely distributed in cereal fields of the province which are of economic importance and are considered serious pests of wheat and barley crops . The prominence of *Pratylenchus neglectus* and *P. thornei* with high population density in soil as well in roots differed from others. Thirty-one percent of collected cyst nematodes were identified as *H. filipjevi* (Dababat et al. 2020; Imren et al. 2021).

This study highlighted the eco-regional distribution of the main genera of *Pratylenchus* (*P. neglectus* and *P. thornei*) and *Heterodera* (*H. filipjevi*) populations in Azerbaijan, Kazakhstan, and Kyrgyzstan. Global complementation among regional or national research programs has proven to be highly beneficial for identifying and deploying germplasm with higher levels of resistance and tolerance to cereal cyst and root-lesion nematodes. However, these global efforts currently lack effective funding and coordination, limiting the ability to realize the benefits already known to exist. Greater collaboration is therefore needed between advanced research institutions, international organizations such as CIMMYT and ICARDA, and scientists in countries where these nematodes are known to be a problem. These collaborative efforts will provide greater understanding of the complexity, economic importance, and control of *Heterodera* and *Pratylenchus* populations, and of pathotype evolution or selection for *Heterodera* species. Such information is necessary to select wheat cultivars carrying the correct resistance genes for nematode control.

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Current status of cereal cyst nematodes *(Heterodera filipjevi)* in Türkiye

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Summary

Several Cereal Cyst Nematodes (CCN) cause severe damages to numerous crops including wheat. Cereal cyst nematode, *Heterodera filipjevi*, is one of the main biotic stresses that cause yield loss in rainfed growing areas of winter wheat. This nematode species is also known to be the most common nematode pest in the winter wheat fields of Türkiye. Another important CCN, *H. avenae* was detected by several researchers in Türkiye. It was also reported that *H. filipjevi*. was found in the Central Anatolian Region of Türkiye. *Heterodera avenae* was detected in the wheat fields of Çukurova and Aegean regions by several researchers. The *H. avenae* and *H. latipons* may found in in the Southeastern Anatolia Region with mixed populations. This review is performed to reveal the presence, impact and current status of *H. filipjevi*, one of the most important species of CCN nematodes in Türkiye.

Introduction

Wheat is one of the most important crops due to its essential role in the human and animal diet, and its importance in food security. Türkiye is the world's top ten producers of wheat and therefore has an important role in global wheat production (FAO 2021). Bread wheat covers 86 % and durum wheat covers 16 % of the total cultivated wheat area in Türkiye (TUIK 2016).

Plant parasitic nematodes (PPNs) are one of the important biotic stresses that cause economical level yield losses in wheat-growing regions. On a global scale, there is a 10% loss from PPNs and 7-10% in wheat production (Sasser 1987; Smiley et al. 2017; Whitehead 1998). Also, it has been reported that yield losses from nematodes in wheat can reach up to 35-40% due to population density and abiotic stress factors especially drought (Williamson and Gleason 2003).

The CCN *H. avenae* was first detected in Erzurum (Yüksel 1974), and then Öztürk et al. (1992) in wheat fields in Konya. Rumpenhorst et al. (1996) reported that the species known as *H. avenae* in the Central Anatolian region is *H. filipjevi*. Subbotin et al. (2003) and Şahin et al. (2009) reported that only *H. filipjevi* was found in the Central Anatolian region. *Heterodera avenae* was detected in the wheat fields of Çukurova region (Gözel 2001; Subbotin et al. 2003) and Aegean region (Mısırlıoğlu 2006). While in the Southeastern Anatolia Region, *H. avenae* and *H. latipons* are found in mixed populations (Abidou et al. 2005; İmren et al. 2010; Kılıç 2011; Öcal 2012), the most common species being *H. latipons*. reported (Imren et al. 2012).

Heterodera filipjevi is identified by forming cysts on root systems. It also causes yellowing on the head of plants and helps in the penetration of other root pathogens. Recent studies showed that *H. filipjevi* causes yield losses of up to 50% in Türkiye (Dababat et al. 2015).

History

The genus *Heterodera* includes 12 species that adversely affect the roots of cereals and grasses. These species of (CCNs) are designated as the *Heterodera avenae* group (Rivoal & Cook, 1993). Among these, three species (*H. avenae, H. filipjevi, and H. latipons*) are the most important cyst nematodes attacking wheat, causing economic yield loss worldwide (Handoo 2002; Subbotin et al. 2003, 2010). *Heterodera filipjevi* which was identified as *H. avenae* (Kirjanova and Krall 1971) formerly was first found in 1964 in cereal fields of Tajikistan and described as a new species by Madzhidov (1981). In later studies, it has been revealed that the previous identification of different *Heterodera* spp. such as *H. avenae* and *H. mani* are actually *H. filipjevi* (Subbotin et al. 1996; Sturhan and Rumpenhorst 1996). Currently, *H. filipjevi* which has at least two pathotypes (Ha22 and Ha33) from the Ha33group is found in many countries in Europe and Asia (Toktay et al. 2012; Dababat et al. 2015, 2017), and in two northwestern states of the U.S. (Smiley 2015). The hosts of *H. filipjevi* are wheat, rye, barley, maize as well as other grass species (Smiley et al. 2017).

In Türkiye, CCNs such as *H. avenae* was first detected in Erzurum (Yüksel, 1974), and then Öztürk et al. (1992) in wheat fields in Konya. Rumpenhorst et al. (1996) reported that the species known as *H. avenae* in the Central Anatolian Region is *H. filipjevi*. In their study in the Central Anatolia region, they reported that the majority of nematodes from 15 cyst samples and 12 juvenile samples were *H. filipjevi*, *H. latipons* in two locations, and *H. avenae* in one location. The most harmful nematode in wheat and barley growing areas is probably *H. filipjevi*, especially in semiarid areas, as there is an increase in nematode damage under drought stress conditions (Rivoal and Cook 1993; Nicol 2002).

Status

Heterodera filipjevi has economic importance on winter wheat in Central and Eastern Anatolian regions of Türkiye, where it causes significant yield losses and can be found in high densities in these areas (Imren et al., 2016; Şahin et al 2009). Toktay et al 2015 reported that 32.50% of wheat field areas were infected with CCNs in addition, the infestation rates were highest (60%) in Elazığ province. A study by Cui et al. (2017) reported that *H. filipjevi* was found in 5 main regions of Türkiye (Central Anatolia, Marmara, Aegean, Southeast Anatolia, and Black Sea Region) from samples collected from wheat and barley fields in 21 districts. As a result of the study carried out in 13 provinces representing the most important wheat production areas of Türkiye in 2018-2019, it was determined that 42.8% of the wheat cultivation areas of the Central Anatolia Region were infested with *H. filipjevi*. The highest infection rate with *H. filipjevi* was detected in Eskişehir (75%), followed by Kırşehir (57.2%), Kırıkkale (56.3%), Konya (54.8%), Kayseri (45.9%), Nevşehir (42.9%), Sivas (37.9%), Yozgat (37%), Niğde (33.4%), Aksaray (26.7%), Ankara (25%), Çankırı (25%) and Karaman (14.3%), respectively (Yatkın et al. 2021).

Impact

With *H. filipjevi* being widely distributed in major wheat and barley cultivation areas, the average yield losses caused by the nematode are estimated to be around 42% for rainfed winter wheat grown in the Central

Anatolian Plateau in the province of Ankara (Nicol et al. 2006) and has a potential to cause damage and yield losses in wheat cultivation in Bolu Province, Türkiye, up to 40.52% (Imren et al. 2020). Also, it was reported in Türkiye that the threshold density that has a negative economic impact on yield due to *H. filipjevi* may be in the range of 5 eggs/g soil (Sahin et al. 2009). While Sahin et al. (2008) reported that *H. filipjevi* has been found to infest wheat and barley crops in several Turkish regions, where the nematode population densities have reached 115 eggs and J2/g soil. In addition, in Yatkın et al. (2021), the highest and lowest egg+J2 of *H. filipjevi* were determined as 104 eggs+J2 g soil and 2 eggs+J2 g soil in Kırşehir/Merkez and Konya/Sarayönü populations, respectively.

One of the most important steps in determining the amount of yield loss brought on by nematode infestation is to analyze the association between the population density of CCNs and wheat production, due to *H. filipjevi* presence in Turkish cereal production systems. However, little is known regarding the relationship between initial population (Pi) densities and wheat yield response (Imren et al. 2020).

Recommendations

Nematode damage is often ignored because they are microscopic and usually do not show any specific symptoms. Also, many farmers are unaware of nematodes and the damage they cause. This situation is more common in cereal production areas. Also, the difficulties in minimizing the damage caused by CCNs are complicated by the refusal of experts to acknowledge CCNs as a significant factor restricting cereal productivity. Because the impact of CCNs on wheat output has not been widely studied, especially in poorer nations, fewer experts are aware of the significance of CCNs (Smiley and Nicol 2009).

The survival of CCN second-stage juvenile in the cyst for many years before hatching is the most important factor that complicates the CCN struggle. The survival of the second-stage juvenile for many years before hatching requires long-term management planning. Nematicides, which are the most important means of control for many nematode species, have very limited use in cereal due to their cost. For these reasons, the use of crop rotation and resistant varieties are the most important management tools that are being practiced. Crop rotation, which is another important management tool, is extremely difficult to apply in dry farming areas such as Türkiye. For this reason, the use of resistant varieties are considered one of the best methods for CCN control. However, the fact that resistant varieties do not provide resistance to all CCN species and races, and that resistance sources from single dominant genes do not provide resistance to other important species such as *Pratylenchus* spp., limit the use of resistant varieties.

Cereal cyst nematodes can be controlled by lowering the population below the economic damage threshold. Definitive research on population dynamics and yield losses on representative local cultivars in natural field settings is necessary for this. CCNs can be efficiently controlled through 1) cultural methods based on rotational combinations of non-hosts such as legumes 2) moderately resistant and resistant cultivars 3) clean fallow. The efficiency and longevity of the resistance gene(s) used against the specific nematode population, as well as a thorough understanding of the virulence and diapause characteristics of the local nematode population, are all required for each of these management measures. There are numerous instances where the use of crop rotations, resistant cultivars, and chemical control techniques have successfully managed CCNs. A focused and integrated approach is the best way to control CCNs. When two or more soil-borne diseases are present in the soil at the same time, integrated management which is mostly based on genetic host resistance appears to be the most effective (Nicol and Rivoal 2008).

Globally, there are several known sources of genetic resistance in wheat against cyst nematodes, however, we have yet to investigate if these genetic sources will be useable in Türkiye. In order to establish this, we need to clearly understand the nematode species and their reactions to known gene(s). The International Maize and Wheat Improvement Centre (CIMMYT) and the joint Soilborne pathogens program (Türkiye CIMMYT ICARDA) have obtained these sources over the past 10 years and have incorporated them into both spring and winter varieties by crossing with high-yielding, adapted wheat for global wheat improvement, which is now in Türkiye. Furthermore, through this international collaboration, CIMMYT has gained access to molecular markers for some of the resistance genes (e.g., *Cre1* and *Cre3*) against *H. avenae* (pathotype Ha13). Throughout the course of this project access to other markers that were developed by Advanced Research Institutions is anticipated and with further assist to the development of cereal cultivars with nematode resistance.

In conclusion, CCN species are widespread in many wheats growing area in Türkiye. Research to further determine economic importance and improve resistance varieties against these pathogens because chemical control is not economical to combat these pathogens.

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Current status of root lesion nematodes (*Pratylenchus* spp.) in Türkiye

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Summary

Root lesion nematodes (RLNs) are one of the most common cereal nematodes causing significant damage to cereals in the globe where cereals grow. They infect more than several hundred of species and may found in temperate, Mediterranean climate, warm, and tropical climate zones. RLNs placed in the *Pratylenchus* genera which have several species such as *Pratylenchus neglectus, P. penetrans, P. zeae, P. coffeae, P. thornei* and *P. vulnus* are some of the most common species in the world. Several species of RLNs are widely distributed in in cereal growing fields in Türkiye. *P. penetrans P. zeae, P. neglectus, P. sefaensis, P. pratensis, P. thornei, P. pratensisobrinus, P. agilis, P. crenatus, P. loosi, P. vulnus, P. delattrei* and *P. mediterraneus* are species of RLNs found by several researchers in Türkiye. These nematodes cause damage to plants at an economic level. As a result of RLNs feeding on the roots of hosts, their water and nutrient intake is limited, and numerous soil-borne pathogens can easily infect through the wounds by the assistance of nematode induced wounds. The status of RLNs in Türkiye, its damage to plants, and some gaps related to RLNs are not fully understood. Therefore, in this review, RLN status, damage and interactions with host plants are presented.

Introduction

Root lesion nematodes are ranked third among the most damaging nematode species after cyst and Root knot nematodes (Jones et al. 2013; Janssen et al. 2017). Root lesion nematodes may infect about 400 plant species and are widely found in tropical, temperate and cool climate zones (Castillo and Vovlas 2007; Bernard et al. 2017). *Pratylenchus neglectus* and *P. thornei* are the predominant species (Maafi et al. 2015).

P. thornei, P. zeae, P. penetrans, P. coffeae P. neglectus and *P. vulnus* are the most common species. Many of the RNLs are polyphagous and mostly reproduce parthenogenetically. RLNs can move between cells in the root and cause damage to the roots as a result of their feeding. When the RLN nematodes feed on the roots, water and nutrient intake is restricted, and many soil-borne pathogens may enter from wounds. A total of 13 species including *P. penetrans, P. thornei, P. delattrei, P. sefaensis, P. vulnus, P. pratensisobrinus, P. zeae, P. agilis, P. pratensis P. loosi, P. crenatus, P. mediterraneus* and *P. neglectus* are found in Türkiye (Kepenekci et al. 2012).

Status and damage of root lesion nematodes

Numerous species (68 species) of *Pratylenchus* genus (Castillo and Vovlas 2007) are found in the world. Several species of this genus are found in cereals fields such as *P. crenatus, P. thornei, P. neglectus* and *P. penetrans* that are distributed in globally (Rivoal and Cook 1993).

Root lesion nematodes may infect many plant species (Castillo and Vovlas 2007; Bernard et al. 2017) may cause damage to trees varies depending on biotic stress factors (Dickerson et al. 2000). *P. coffeae* causes a growth retardation of up to 80% in citrus fruits (O'Bannon and Tomerlin 1973) and *P. penetrans* causes growth retardation at 30 individuals/100 cm^3 soil density in pear, 80 individuals/100 cm^3 soil density in cherry and 320 individuals/100 cm^3 soil density in plum (Nyczepir and Halbrendt 1993). In addition, root lesion nematode species causes up to 75% damage on peach trees at soil densities in 457 individuals/100 cm^3 (Nyczepir and Halbrendt 1993).

Wheat is an important food crop for many countries and plays important roles in food grain production in the world. Wheat has an important place in human nutrition. Wheat is cultivated on an area of 219006893 hectares in the world and 260925831 tons of product is obtained and the average yield is 34744 kg/ha (FAO 2020). In Türkiye, wheat is planted on 6914632 hectares, the average yield is 29647 kg/ha, with a total production of 20500000 tons (FAO 2020).

Worldwide crop losses caused by nematodes are estimated at more than a hundred billion dollars per year (Abad et al. 2008). In addition to other plant parasitic nematode genera, the root lesion nematodes, the *Pratylenchus* genera, may cause significant yield losses (Rivoal and Cook 1993).

P. thornei is a major species in both wheat and barley fields (Ahmedi et al. 2015). Root lesion nematodes are found in 35% in wheat fields and 42% of barley fields (Ahmedi et al. 2015). Population densities of root lesion nematodes ranged from 1 to 365 nematodes/g root in wheat fields and 0 to 1334 nematodes/250 cm^3 in barley (Ahmedi et al. 2015).

The root lesion nematodes are identified using molecular (Waeynberg et al. 2000), morphological and morphometric characters (Loof 1991). The distributed rate of plant parasitic nematodes genera: *Pratylenchus* (73.3%), *Tylenchorhynchus* (37.3%), *Helicotylenchus* (38.8%), *Paratylenchus* (26.6%), *Pratylenchoides* (66.6%), *Merlinius* (78.6%), and *Heterodera* (82.6%) are commonly found in Bolu province of Türkiye (Imren et al. 2015).

More than 8 species of *Pratylenchus* are parasitic on small grain cereals (Nicol 2002; Nicol et al. 2003; McDonald and Nicol 2005; Castillo and Vovlas 2007) and P. *thornei, P. crenatus, P. penetrans* and *P. neglectus* are found as an economically important root lesion nematodes in temperate cereal-producing regions in the world (McDonald and Nicol 2005). *Pratylenchus* species is one of the most economically damaging nematodes for cereals in the world (Nicol et al. 2011; Duncan and Moens 2006). Root lesion nematodes cause crop losses up to A$ 73 million in Australia (Meagher et al. 2015; Murray and Brennan 2009).

P. *thornei, P. fallax, P. neglectus, P. penetrans* and *P. crenatus* are found in barley and wheat growing regions including the Central Anatolia Region, Eastern Mediterranean Region and South East Anatolian Region of Türkiye (Kılıc et al. 2012; Imren and Elekcioğlu 2008; Ocal 2012; Sahin et al. 2009).

P.neglectus and *P. thornei* exist in 82% of the wheat fields (Tanha Maafi et al. 2009). *P. penetrans* is the most plentiful and prevalent species in Morocco in wheat growing fields (Mokrini et al. 2012). The population density and frequency of *Pratylenchus* species in cereals fields were found in different localities where host plants grow (Riley et al. 2002). Among the root lesion nematodes *P. neglectus* is the most significant and widely distributed in the western and southern regions of Australia (Vanstone et al. 2008, Meagher et al. 2015, Riley and Kelly 2002).

The yield losses caused by root lesion nematodes ranged from 19-70% in Türkiye (Toktay et al. 2015). *P. thornei* and *P. neglectus* are common species in Erzurum, Igdir, Kars, Elazig, Erzincan, Sivas and Malatya provinces in wheat fields of Türkiye (Toktay et al. 2015). *P. neglectus* and *P. thornei* are found in 43% of samples in the Central Anatolian Region of Türkiye (Şahin et al. 2009).

Crop rotation is a favored in management practice, limiting damage by RLNs species through rotation, however *Pratylenchus* species infect many host plant species (Talavera and Vanstone 2001; Vanstone and Russ 2001). *P. neglectus* and *P. thornei* are most important wheat nematodes in Türkiye, and national wheat varieties and wild landraces are moderately resistant to those species (Kasapoglu et al. 2015). Development of tolerant and locally adapted varieties is essential for preventing damage by RLNs population densities and are influenced by cropping systems (Gitti et al. 2015, Riley et al. 2002). Nematicide application on wheat decreases the RLNs population up to 92% and increases the yield of grain by 22.3% (Gitti et al. 2015).

Some resistances are available against *P. thornei* in cereal in Australia (Sheedy and Thompson 2009). Possibly six genes involve in resistance against to *P. thornei* (Thompson and Seymour 2011; Meagher et al. 2015). The resistance presents to *P. neglectus* in 6DS and 2BS chromosomes and 6DL, 6DS, 2BS chromosomes to *P. thornei* (Zwart et al. 2010; Meagher et al. 2015). In addition, RNA interference gene silencing reveals that the reproduction nematodes decrease at 80% when target genes are silenced (Tan et al. 2013; Meagher et al. 2015).

Conclusion

The genetic resistance to root lesion nematodes in cereals genotypes and varieties are important. Türkiye has a diverse climate conditions and contains landraces that probable contain resistance genes of cereals against to root lesion nematodes more than was once thought. Comprehensive studies lead to determine management strategies by seeking resistance sources in Turkish cereal landraces and commercial cultivars by using both phenotypic screening and molecular markers. The economic importance of the some common species of root lesion nematodes has not been fully understood. Therefore, determining and researching intensively on crop losses, distribution, biology of common and economically important species of root lesion nematodes are decisively important.

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Current status of cereal cyst nematodes (*Heterodera* spp.) in wheat and barley fields in Syria

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Summary

Cereal cyst nematodes (CCN) are important plant-parasitic nematodes of wheat and barley, and exist in most of the cereal growing regions of the world. As there is limited information on the nature and distribution of CCN species in Syria, a survey was conducted in north-eastern Syria to assess the distribution of CCN in the main wheat and barley growing areas. In the summer of 2009, a total of 167 composite soil samples were collected from 167 wheat and barley fields. Cysts were extracted from soil using the Fenwick can technique, then quantified and identified up to the species level by using both morphological and molecular methods. The study revealed that 62% of the fields were infested with the three *Heterodera* species: *H. avenae*, *H. filipjevi* and *H. latipons*. However, the most prevalent species was *H. latipons*, which was present in 76% of the infested samples, while it occurred alone in 67% of those samples. *Heterodera avenae* was detected singly in 20% of the samples, while 11% consisted of mixed populations with other species. *Heterodera filipjevi* was never detected alone; it was found mixed with *H. avenae* and/or *H. latipons* in 9% of the samples. The high number of extracted cysts (up to 116 cysts / 200 g of soil) is most likely related to the monoculture practiced in that region and the fact that growers are unaware of the existence of resistant varieties. Currently, almost all research is stopped due to the difficult situation of funding and the spread of bombs and explosives in cereals fields.

Keywords: cereals, *Heterodera avenae, H. filipjevi, H. latipons,* survey.

Introduction

Heterodera avenae, *H. filipjevi* and *H. latipons*, the three major species of the Avenae group are common in Türkiye, a country adjacent to Syria. However, the dominant species varies with the region. *Heterodera avenae* and *H. latipons* were found in the south-eastern and eastern Mediterranean region, whereas *H. filipjevi* was prevalent in the Central Anatolian Plateau region (Öztürk et al. 1998; Abidou et al. 2005; Dababat et al. 2014). The three species were also found in cereal fields in Syria (Rivoal et al. 2003; Abidou et al. 2005; Hassan et al. 2010); they are widely distributed, with *H. latipons* being dominant (Scholz 2001).

Wheat and barley growing areas are distributed all over Syria. The annual wheat yield ranges between 3 and 4.5 million tonnes (Anonymous 2010). However, after the year 2011, wheat production in Syria declined significantly, and during 2012 Syria was forced to import flour for the first time, and more than half of the wheat-producing areas in the north and south of the country were out of production. Referring to the economic importance of wheat, increasing concerns about the severity of the damage caused by CCN, and the little-known information about the distribution of the three species of CCN in Syria, the objectives of this study were: (1) to determine the current prevalence, incidence and geographical distribution of CCN in the north-eastern region of Syria, and (2) to evaluate their infestation levels and to compare the obtained data with those from previous surveys carried out in the country. The acquired knowledge should guide the development of the nematological research programme for cereals.

Methods

Sampling

The survey was performed immediately after the harvest of cereals in 2009. Soil samples were taken in 167 fields (Table 1) spread over five of the major cereal-growing provinces (Aleppo, Idlib, El-Raqqa, Deir Ez Zur and El-Hassake; Figure 1). Ninety-five samples were taken from rain-fed (40 samples) and irrigated wheat fields (55 samples); the remaining 72 samples were obtained from barley fields (54 rain-fed and 18 irrigated).

Figure 1. Map of Syria showing sampling areas for cereal cyst nematodes (*Heterodera* spp.).

Cyst extraction and nematode identification

For each sample, cysts were extracted (Fenwick, 1940) from three 200 g subsamples. Extracted cysts were retained on a 250-µm sieve. For each sample, the vulval cone of 2-5 mature cysts was mounted in glycerine jelly. Identification of *Heterodera* species was based on the shape of the cyst, vulval slit, underbridge structure, and presence or absence of bullae (Handoo 2002). Samples with fewer cysts were identified molecularly only

(Ferris et al. 1993; Toumi et al. 2013a,b). Molecular identification was restricted to about 50% of the samples from each province, because of financial restrictions.

Results

The morphological observations resulted in the identification of three species (Table 1). Lemon-shaped cysts with a short vulval slit, clear and crowded bullae, and no underbridge in the vulval cone were identified as *H. avenae*. Cysts of *H. filipjevi* were also lemon-shaped, had big bullae, and a distinct underbridge, thick in the middle and thin at the ends. *Heterodera latipons* cysts were lemon-shaped, and showed a short vulval slit, no bullae and a very strong underbridge with a pronounced enlargement in the middle (Figure 2). Amplification of the rDNA-ITS region was successful for all selected samples; sequencing of both DNA strands of the PCR products confirmed the morphological identification of corresponding samples. According to the results of both morphological and molecular identifications, *H. latipons*, *H. avenae* and *H. filipjevi* were present in 62% of the 104 fields surveyed in the region (Figure 4). *Heterodera latipons* was the most prevailing species in wheat and barley fields throughout the surveyed sites. The highest population density was found in El-Raqqa (116 cysts (200 g)$^{-1}$ soil). All fields in El-Raqqa and Idlib were infested only with *H. latipons* (Figure 5). *Heterodera avenae* was found in 32 fields (31% of the infested fields). The highest incidence was in Aleppo (53 cysts (200 g)$^{-1}$). *Heterodera avenae* occurred singly in 21 fields (20%) and mixed with *H. latipons* or *H. filipjevi* in 11 fields (11%) (Figure 4). *Heterodera filipjevi* occurred only in El-Hassake in 9% of infested fields (9 fields) always in a mix with *H. latipons* and/or *H. avenae* (Figure 4). High infestation levels of nematodes were found in barley fields (69% of the fields). However, only in three provinces out of the five surveyed, the infestation levels in barley fields were higher than in wheat fields: Aleppo (91%), Deir Ez Zur (56%) and El-Raqqa (43%) (Figure 6).

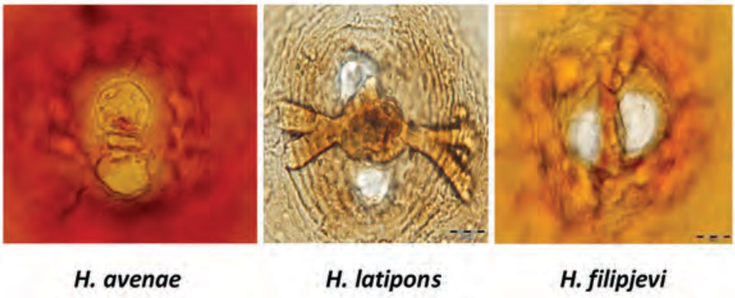

Figure 2. Vulval cone of the three species of cereal cyst nematodes detected during the survey.

Discussion

This survey demonstrates that the major cereal growing areas in Syria are commonly infested by three species belonging to the Avenae group. As was reported earlier (Scholz 2001; Abidou et al. 2005; Hassan 2008) *H. latipons* is the most widely distributed species and occurs in all regions. Heavy infestations of cyst nematodes, especially *H. latipons*, were found most commonly in barley fields in the dry areas of Syria (< 300 mm annual rainfall). In the last decade, because of the increase in the incidence of drought conditions, farmers prefer to grow barley instead of wheat because, unlike wheat, barley can be grown with limited rainfall. The monoculture of barley further aims at a higher yield to feed a larger number of animal stock (Scholz 2001; Nicol et al. 2004).

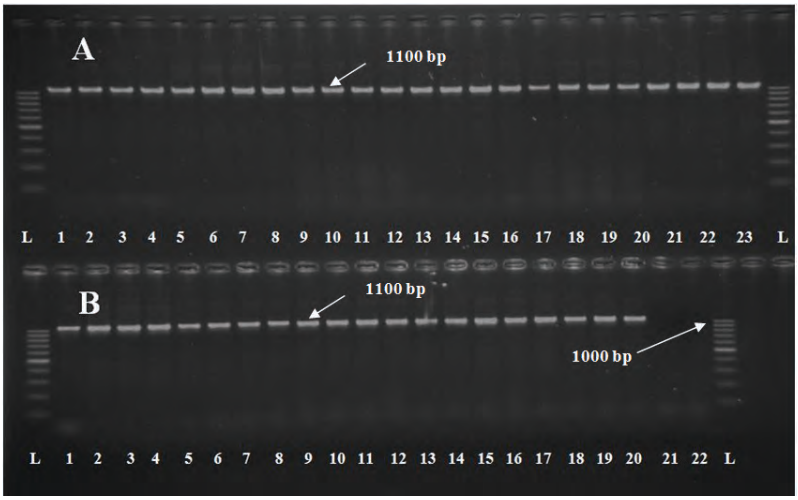

Figure 3. Selected results of the universal PCR of ITS regions (rDNA) of Heterodera isolates taken in five Syrian provinces (Aleppo, Idlib, El-Raqqa, Deir Ez Zur and El-Hassake).

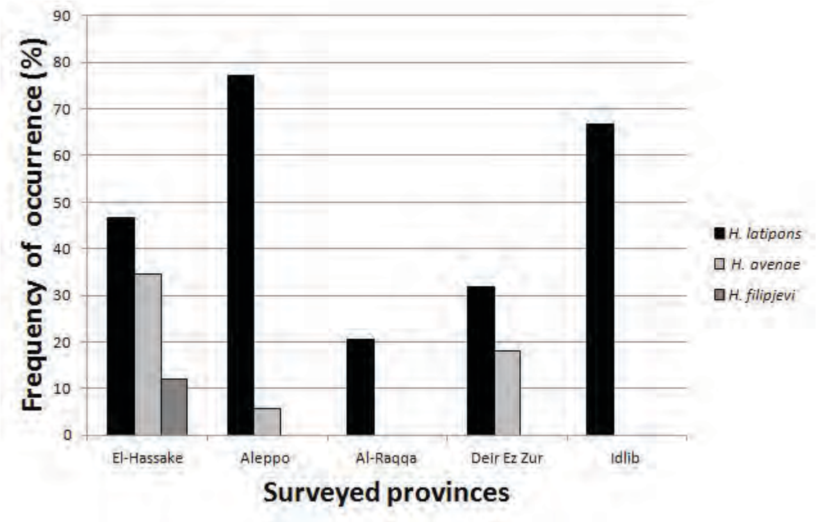

Figure 4. Occurrence of three species of cereal cyst nematodes in the five Syrian provinces surveyed.

The survey also shows that *H. avenae* and *H. filipjevi* are more widespread than previously reported (Scholz 2001; Abidou et al. 2005; Hassan 2008). Abidou et al. (2005) described *H. filipjevi* from only one location, whereas in this survey, the species was detected at nine locations close to the Turkish border. This result suggests a further extension of the spread of *H. filipjevi* in Syria and Türkiye (Imren et al. 2012). *Heterodera filipjevi* is common in temperate climates in many European countries; the East European region is considered to be the centre of distribution of the species. The areas we found infested with *H. filipjevi* are somehow similar in climate to the center of distribution of *H. filipjevi*, as they were situated at higher altitudes with lower temperatures and more rainfall than areas where *H. avenae* and *H. latipons* were detected abundantly. These findings confirm the differences in the ecoregional distribution of the three major species of CCN and their link with the geographic and climate difference, in addition to the suggested early divergence between tropical and temperate heteroderid species. It would be worth developing some phylogeography approaches to establishing the principles and processes determining the geographical distribution of these differentiation lineages. Fields in the other four provinces (Aleppo, Idlib, El-Raqqa and Deir Ez Zur) were not found infested with *H. filipjevi*. Additionally, all fields in El-Raqqa and Idlib were free of *H. avenae*. However, due to the relatively low number of collected samples compared with the wide area surveyed, the strict outcome of this investigation regarding the negative samples (*e.g.* one or two species do not exist in a province) may not reflect the accurate occurrence of CCN. Therefore, an intensive survey of CCN distribution is still required in those provinces.

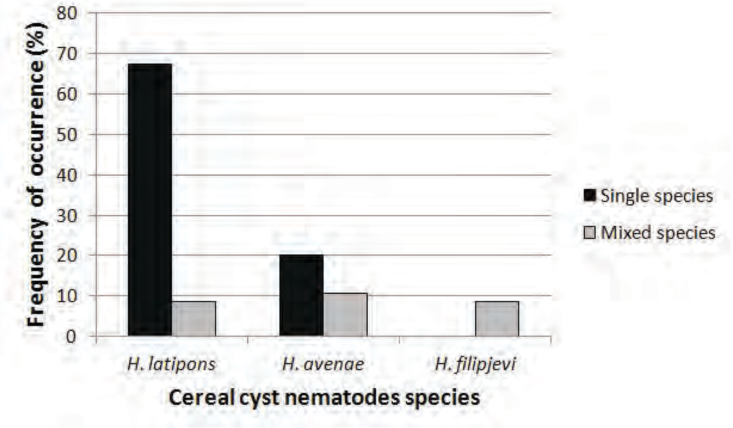

Figure 5. Occurrence of three species of cereal cyst nematodes, single or mixed with each other.

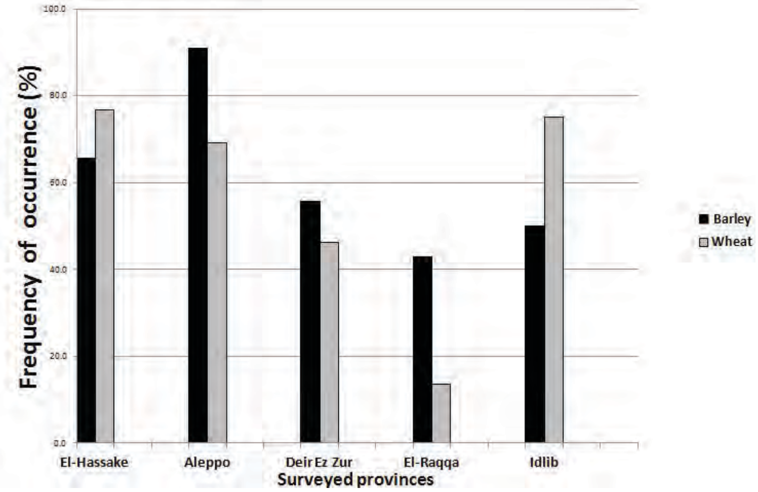

Figure 06. Occurrence of cereal cyst nematodes according to the host (barley - wheat) in five Syrian provinces.

The morphological identification of the collected cysts was not straightforward because of the presence of species mixtures. Moreover, in some samples, the number of cysts was low and not enough to allow morphological identification. Hence, identification via sequencing was used. However, sequencing is relatively expensive, and time-consuming, and because of the large number of samples, approximately half the number of samples had to be sequenced. Subsequent species-specific PCR assays enabled fast and accurate identification.

The wide distribution of CCN species in the surveyed regions and the high numbers of cysts in some samples is probably related to: 1) the monoculture of cereals practised in these regions, where the damage caused by the nematodes is likely to be greater as well (Ibrahim et al. 1999, Smiley and Nicol 2009), 2) the fact that farmers are unaware of the existence of resistant varieties, 3) the strong wind in the north-eastern regions in Syria responsible for the aerial spread of soil and cysts to other locations, 4) an irrigation system which is still not well developed but allows water to carry cysts to lower situated fields, and 5) non-use of agricultural practices such as clean fallow, weed control and cleaning machinery. Research by Kerry (1982) showed that after many years of monoculture of cereals, *H. avenae* populations declined due to the increase in the density of the fungal parasites that totally destroyed females on roots or resulted in the formation of small cysts, which are often empty. It is worth observing whether this phenomenon also happens in Syria and what the outcome might be.

To maintain the population densities of cereal cyst nematodes below damaging levels, appropriate management measures such as crop rotation and the use of resistant varieties, are necessary. A number of resistance sources for breeding purposes have been detected in domestic cereals and their wild relatives, acting against both *H. avenae* and *Pratylenchus* spp. (Nicol et al. 2004). Preliminary studies indicate that several resistance genes of barley or wheat are, to some extent, also active against populations of *H. filipjevi* or *H. latipons* originating from different sites in North Africa, Europe and Asia (Bekal et al. 1998; Rivoal et al. 2001; Mokabli et al. 2002). Further efforts should be made to characterise existing varieties and identify resistance amongst existing cultivars as well as new sources to be used in cereal breeding programmes.

To develop a long-term strategy for CCN management, cultivars from other countries or from the local breeding programme should be screened for resistance to the prevailing CCN species. Also, comprehensive and regional surveys in Türkiye, Syria, Lebanon and Jordan are needed for establishing the mapping population densities in order to define potential economic damage rates and determine more accurately the distribution of CCN. Also, to increase the understanding of the geographical distribution of CCN, and clarify the relation with different hosts and different cultivars of barley or wheat. However, However, due to the war, many fields were out of use due to the spread of bombs and explosives in fields. Hence, many farmers left their fields, and that resulted in a shortage of wheat production and yield since 2011, and the year 2022 we had less than one million tons of wheat, while our need is almost 1.7-2 million tons. hopefully, in the coming days will be safer to be able to get back to our field safely and restart the production as before the war.

Table 1. Results of the soil sampling for cereal cyst nematodes in fields ofthe five major cereal growing provinces of the north-eastern region of Syria including the negative samples

Code	Province	Village	Host	Irrigation	Number of cysts (200 g soil)$^{-1}$	Species
GH100	Aleppo	El-Shiokh Fokani	barley	rain-fed	1	*H. latipons* (Hl)
GH103	Aleppo	Mhajan	barley	rain-fed	8	Hl
GH108	Aleppo	Maran	barley	rain-fed	5	*H. avenae* (Ha)
GH110	Aleppo	Biaat El-Deniesh	barley	rain-fed	53	Ha
GH76	Aleppo	Thalthana	barley	rain-fed	11	Hl
GH77	Aleppo	Thalthana	barley	rain-fed	44	Hl
GH78	Aleppo	Aghtarien	barley	rain-fed	23	Hl
GH79	Aleppo	Aghtarien	barley	rain-fed	20	Hl
GH80	Aleppo	Hazwan	barley	rain-fed	1	Hl
GH81	Aleppo	Hazwan	barley	rain-fed	6	Hl
GH82	Aleppo	Hadadien	barley	rain-fed	24	Hl
GH84	Aleppo	Atien	barley	rain-fed	33	Hl
GH85	Aleppo	Atien	barley	rain-fed	55	Hl
GH86	Aleppo	El-Dana	barley	rain-fed	51	Hl
GH87	Aleppo	El-Dana	barley	rain-fed	9	Hl
GH88	Aleppo	El-Bab	barley	rain-fed	0	-*
GH90	Aleppo	El-Naziha	barley	rain-fed	40	Hl
GH91	Aleppo	El-Naziha	barley	rain-fed	19	Hl
GH92	Aleppo	Halab	barley	rain-fed	0	-
GH96	Aleppo	Brida	barley	rain-fed	1	Hl
GH97	Aleppo	Brida	barley	rain-fed	37	Hl
GH98	Aleppo	Moman	barley	rain-fed	1	Hl
GH101	Aleppo	El-Shiokh Fokani	wheat	rain-fed	1	Hl
GH102	Aleppo	Anadan	wheat	irrigated	4	Hl
GH104	Aleppo	El-Reshaf	wheat	rain-fed	7	Hl
GH105	Aleppo	Hwar el-Nahr	wheat	rain-fed	14	Hl
GH106	Aleppo	Der El-Jemal	wheat	irrigated	12	Hl
GH107	Aleppo	Marea	wheat	irrigated	24	Hl
GH109	Aleppo	Nobel	wheat	irrigated	21	Hl
GH83	Aleppo	Hadadien	wheat	irrigated	0	-
GH89	Aleppo	El-Bab	wheat	rain-fed	0	-
GH93	Aleppo	Halab	wheat	irrigated	0	-
GH94	Aleppo	Tel Refat	wheat	rain-fed	38	Hl
GH95	Aleppo	Tel Refat	wheat	rain-fed	5	Hl

GH99	Aleppo	Moman	wheat	irrigated	0	-
GH141	Deir Ez Zur	Hawie El-Maiadien	barley	irrigated	0	-
GH146	Deir Ez Zur	El-Tiba	barley	irrigated	3	Hl
GH147	Deir Ez Zur	El-Tiba	barley	irrigated	6	Hl
GH150	Deir Ez Zur	Bilaoom	barley	irrigated	10	Hl
GH151	Deir Ez Zur	Toob	barley	irrigated	0	-
GH154	Deir Ez Zur	Shakra	barley	rain-fed	5	Hl
GH156	Deir Ez Zur	Hsikia	barley	irrigated	7	Hl
GH160	Deir Ez Zur	Sealo El-Ankshi	barley	irrigated	0	-
GH161	Deir Ez Zur	Sealo El-Mawkiea	barley	irrigated	0	-
GH140	Deir Ez Zur	Hawie Mohsen	wheat	irrigated	4	Hl
GH142	Deir Ez Zur	El-Zebarie	wheat	irrigated	9	Ha
GH143	Deir Ez Zur	El-Zebarie	wheat	irrigated	6	Ha
GH144	Deir Ez Zur	El-Swieaiea	wheat	irrigated	0	-
GH145	Deir Ez Zur	El-Swieaiea	wheat	irrigated	5	Ha
GH148	Deir Ez Zur	Bolil	wheat	irrigated	0	-
GH149	Deir Ez Zur	Bolil	wheat	irrigated	0	-
GH152	Deir Ez Zur	Mohkan	wheat	irrigated	0	-
GH153	Deir Ez Zur	Morad	wheat	irrigated	8	Hl
GH155	Deir Ez Zur	Jeaiea	wheat	irrigated	0	-
GH157	Deir Ez Zur	Mohmadia	wheat	irrigated	0	-
GH158	Deir Ez Zur	Hatla	wheat	irrigated	0	-
GH159	Deir Ez Zur	Mriaia	wheat	irrigated	12	Ha
GH1	El-Hassake	Tel El-bazri	barley	rain-fed	1	Hl
GH10	El-Hassake	Rhaia	barley	irrigated	2	Hl
GH13	El-Hassake	Sibat	barley	rain-fed	24	Ha
GH14	El-Hassake	Sibat	barley	irrigated	14	Ha
GH17	El-Hassake	El-baghoz	barley	rain-fed	0	-
GH18	El-Hassake	El-baghoz	barley	rain-fed	0	-
GH2	El-Hassake	Tel El-bazri	barley	irrigated	2	Ha
GH20	El-Hassake	Khanamia	barley	rain-fed	10	Hl
GH27	El-Hassake	Boor saied	barley	rain-fed	0	-
GH3	El-Hassake	Khazna	barley	rain-fed	3	Hl
GH33	El-Hassake	Aloni	barley	rain-fed	0	-
GH34	El-Hassake	Aloni	barley	irrigated	0	-
GH35	El-Hassake	Twini	barley	irrigated	0	-
GH36	El-Hassake	Twini	barley	irrigated	2	Ha
GH37	El-Hassake	Tel Aswad	barley	rain-fed	41	Ha
GH38	El-Hassake	Tel Aswad	barley	rain-fed	48	Ha

GH39	El-Hassake	Tel Ailol	barley	rain-fed	35	Hl – Ha – *H. filipjevi* (Hf)
GH4	El-Hassake	Khazna	barley	rain-fed	17	Ha
GH40	El-Hassake	Tel Ailol	barley	rain-fed	27	Hl – Ha – Hf
GH41	El-Hassake	Tel Shaalan	barley	rain-fed	0	-
GH42	El-Hassake	Tel Shaalan	barley	rain-fed	0	-
GH5	El-Hassake	Kherbt El-khder	barley	rain-fed	1	Ha
GH57	El-Hassake	Tel Baidar	barley	rain-fed	0	-
GH6	El-Hassake	Kherbt El-khder	barley	rain-fed	0	-
GH65	El-Hassake	Am Kahfa	barley	rain-fed	60	Hl
GH66	El-Hassake	Am Roos	barley	irrigated	29	Ha
GH68	El-Hassake	Tel Tawiel	barley	irrigated	18	Ha
GH7	El-Hassake	Kherbt El-tamimi	barley	rain-fed	0	-
GH70	El-Hassake	El-Mazar	barley	rain-fed	36	Ha – Hl
GH71	El-Hassake	Zaher El-Arab	barley	irrigated	20	Hl
GH75	El-Hassake	El-bihera	barley	rain-fed	6	Hl
GH8	El-Hassake	Kherbt El-tamimi	barley	rain-fed	1	Hl
GH11	El-Hassake	Safia	wheat	irrigated	14	Hl
GH12	El-Hassake	Safia	wheat	rain-fed	0	-
GH15	El-Hassake	Khanka	wheat	irrigated	3	Ha – Hf
GH16	El-Hassake	Khanka	wheat	rain-fed	5	Hl
GH19	El-Hassake	Khanamia	wheat	rain-fed	27	Hl
GH21	El-Hassake	Salam Aliek	wheat	irrigated	14	Hl
GH22	El-Hassake	Salam Aliek	wheat	irrigated	3	Hl
GH23	El-Hassake	Tel-Skra	wheat	irrigated	0	-
GH24	El-Hassake	Tel-Skra	wheat	irrigated	0	-
GH25	El-Hassake	Jolbsan	wheat	rain-fed	5	Hl
GH26	El-Hassake	Jolbsan	wheat	irrigated	12	Ha – Hf
GH28	El-Hassake	Boor saied	wheat	rain-fed	0	-
GH29	El-Hassake	Kerkwi	wheat	rain-fed	16	Ha
GH30	El-Hassake	Kerkwi	wheat	rain-fed	18	Ha
GH31	El-Hassake	Jatal	wheat	irrigated	6	Hl – Ha
GH32	El-Hassake	Jatal	wheat	rain-fed	11	Hl – Ha
GH43	El-Hassake	Tel Khanzer	wheat	rain-fed	2	Hl
GH44	El-Hassake	Tel Khanzer	wheat	rain-fed	0	-
GH45	El-Hassake	Aamer	wheat	irrigated	11	Hl
GH46	El-Hassake	Aamer	wheat	rain-fed	0	-
GH47	El-Hassake	El-Hatimia	wheat	rain-fed	3	Hl
GH48	El-Hassake	El-Hatimia	wheat	rain-fed	0	-

GH49	El-Hassake	Bripha	wheat	rain-fed	12	Hl
GH50	El-Hassake	Bripha	wheat	rain-fed	3	Hl – Hf
GH51	El-Hassake	Ker Hsar	wheat	rain-fed	6	Hl
GH52	El-Hassake	Ker Hsar	wheat	irrigated	0	-
GH53	El-Hassake	Nes Tel	wheat	irrigated	1	Ha
GH54	El-Hassake	Nes Tel	wheat	rain-fed	0	-
GH55	El-Hassake	Damkhia El-Sakira	wheat	rain-fed	31	Hl
GH56	El-Hassake	Damkhia El-Sakira	wheat	irrigated	9	Ha
GH58	El-Hassake	Tel Baidar	wheat	rain-fed	20	Hl
GH59	El-Hassake	Jriba	wheat	irrigated	5	Ha – Hf
GH60	El-Hassake	Jriba	wheat	rain-fed	12	Ha – Hf
GH61	El-Hassake	Tel Khaled	wheat	irrigated	1	Hl
GH62	El-Hassake	Tel Khaled	wheat	rain-fed	17	Hl
GH63	El-Hassake	Rbieat	wheat	irrigated	3	Hl
GH64	El-Hassake	Tel Fares	wheat	rain-fed	8	Hl
GH67	El-Hassake	Himo	wheat	rain-fed	5	Hl
GH69	El-Hassake	Taalki	wheat	rain-fed	3	Ha
GH72	El-Hassake	Khass	wheat	irrigated	12	Hl – Ha – Hf
GH73	El-Hassake	Hawashia	wheat	rain-fed	22	Hl – Ha
GH74	El-Hassake	El-Karama	wheat	rain-fed	14	Hl – Hf
GH9	El-Hassake	Rhaia	wheat	irrigated	0	-
GH117	El-Raqqa	Moshahed	barley	rain-fed	1	Hl
GH118	El-Raqqa	Moshahed	barley	rain-fed	0	-
GH121	El-Raqqa	Bo Asii	barley	rain-fed	116	Hl
GH125	El-Raqqa	Dkhailieb	barley	rain-fed	9	Hl
GH129	El-Raqqa	Tina	barley	rain-fed	0	-
GH130	El-Raqqa	Tina	barley	rain-fed	0	-
GH137	El-Raqqa	Hafiat El-Wahab	barley	rain-fed	0	-
GH111	El-Raqqa	Kesrat El-shikh Jomaa	wheat	irrigated	0	-
GH112	El-Raqqa	Kesrat El-shikh Jomaa	wheat	irrigated	0	-
GH113	El-Raqqa	Tel El-Saman	wheat	irrigated	0	-
GH114	El-Raqqa	Tel El-Saman	wheat	irrigated	0	-
GH115	El-Raqqa	Tel El-Saman	wheat	irrigated	0	-
GH116	El-Raqqa	Tel El-Saman	wheat	irrigated	0	-
GH119	El-Raqqa	El-Wahda	wheat	irrigated	0	-
GH120	El-Raqqa	El-Wahda	wheat	irrigated	0	-
GH122	El-Raqqa	Bo Asii	wheat	rain-fed	19	Hl
GH123	El-Raqqa	Hzima	wheat	irrigated	0	-

GH124	El-Raqqa	Hzima	wheat	irrigated	0	-
GH126	El-Raqqa	Dkhailieb	wheat	rain-fed	13	Hl
GH127	El-Raqqa	Aien Isa	wheat	rain-fed	0	-
GH128	El-Raqqa	Aien Isa	wheat	rain-fed	5	Hl
GH131	El-Raqqa	El-Raqqa	wheat	irrigated	0	-
GH132	El-Raqqa	El-Raqqa	wheat	irrigated	0	-
GH133	El-Raqqa	Baas	wheat	irrigated	0	-
GH134	El-Raqqa	Baas	wheat	irrigated	0	-
GH135	El-Raqqa	El-Yarmook	wheat	irrigated	0	-
GH136	El-Raqqa	El-Yarmook	wheat	irrigated	0	-
GH138	El-Raqqa	Mazrat Kirtaj	wheat	rain-fed	0	-
GH139	El-Raqqa	El-Asadia	wheat	irrigated	0	-
GH162	Idlib	Freka	barley	irrigated	9	Hl
GH163	Idlib	Jeser Shokor	barley	rain-fed	0	-
GH164	Idlib	Jeser Shokor	wheat	irrigated	0	-
GH165	Idlib	Meles	wheat	rain-fed	11	Hl
GH166	Idlib	Tel Khazal	wheat	rain-fed	4	Hl
GH167	Idlib	Mart Mesrien	wheat	rain-fed	1	Hl

***(-):** Negative samples.

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Overview of seasonal variation of field populations of *Heterodera filipjevi*, *Pratylenchus thornei* and *P. neglectus* on winter wheat in Bolu, Türkiye

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Summary

The development of cereal cyst nematode *Heterodera filipjevi*, root lesion nematodes *Pratylenchus thornei* and *P. neglectus* were investigated under rainfed cereal conditions over three growing seasons (2018-2020) on the winter wheat cultivar Seri-82. Juvenile emergence of *H. filipjevi* was recorded during the winter period from November to March. The hatching process was correlated with the lowest temperature. Mature white females were found on roots at the beginning of May and cysts appeared later on. The total number of cysts and eggs in the soil had only one peak at the end of each growing season, proving the monocyclic nature of *H. filipjevi*. Multiplication rates were inversely correlated with initial nematode densities with ceiling levels of between 15 and 20 eggs per g of dry soil. Population densities of *P. thornei* and *P. neglectus* were low from November to March/April during the cold snow period, increased gradually to June/July and then rapidly decreased over the summer period. Numbers of nematodes were positively correlated with temperature and the multiplication rate was 0.42-3.8 for *P. thornei* and 0.91-2.26 for *P. neglectus*.

Introduction

Wheat (*Triticum aestivum* L.) is cultivated worldwide and is the second most important (http://faostat.fao.org) and is a major staple food in Türkiye (http://turkstat.gov.tr). Wheat is vulnerable to numerous plant pathogens that adversely affect its production (Nicol 2002). Cereal cyst nematodes (CCN) and Root Lesion nematodes (RLN) are a group of root-feeding plant-parasitic nematodes that infect many kinds of cereals used as food crops and are known to be a major constraint to wheat production (Nicol 2002; Dababat et al. 2014).

Root-lesion nematodes (*Pratylenchus* spp.) are widespread and one of the most important groups of plant-parasitic nematodes due to their wide host ranges and their worldwide distribution (Jones et al. 2013). The genus comprises 75 nominal species (Zamora-Araya et al. 2016), with *Pratylenchus thornei* and *P. neglectus* being two of the most frequently reported species (Riga et al. 2008; Yan et al. 2008). In Bolu Province, *Pratylenchus thornei* and *P. neglectus* were recorded in different cereal-producing regions, representing northern Karadeniz Region in Türkiye (Dababat et al. 2019). The cyst nematode, *Heterodera* spp. are important obligate parasites and an economically relevant group of harmful plant parasitic nematodes which are found world-wide (Subbotin et al. 2010 a,b). The main species parasitizing cultivated wheat are *Heterodera avenae*, *H. filipjevi*, *H. hordecalis* and *H. latipons* (McDonald and Nicol 2005). *Heterodera filipjevi* is the most damaging pest that limits cereals production in the major cereal-producing regions of the world including the Central and West Asia (Dababat and Fourie 2018). In Bolu provinces, *H. filipjevi* was first reported by Imren et al. (2018). More recently, this species was recorded in different cereal-producing regions of Marmara region (Keçici et al. 2022).

Cereal nematodes populations can also be managed via crop rotation or cultivation with nonhost crops and fallows. However, nematode biology and population changes should be monitored for effective management, especially under natural field conditions (Thompson et al. 2010). This study was conducted to investigate the seasonal variation of Root lesion nematodes; *P. thornei* and *P. neglectus* and Cyst nematodes *H. filipjevi* in wheat-growing areas in Bolu Province.

Methods

The population dynamics of *H. filipjevi*, *P. thornei* and *P. neglectus* were investigated in a naturally infested experimental field located at Yenicepınar village (40° 45′ 32″ N; 31° 45′ 07″ E) in Bolu. The experimental area is defined as continental, wet and cold in winter, hot and dry in summer. The main climatic features of the study area is defined with average winter and summer months temperatures between 3.5 and 15.5 °C respectively, with average annual precipitation of 550 mm, and an accumulated temperature of 2776 °C (≥ 0 °C). In both growing seasons the experiments were carried out in the beginning of November (http://www.mgm.gov.tr/en-us/about.aspx).

In the study, *H. filipjevi* was identified as the only CCN group species present in the research area, based on morphological and molecular PCR/RFLP identification of the cysts (Imren et al. 2020). Identification of *P. thornei* and *P. neglectus* was made morphologically (Imren et al. 2020). Experiments were conducted during the 2014–15 and 2015–16 wheat-growing seasons, on wheat cultivar Seri-82. The experiment was set up in a randomised block design with seven replications. Soil temperature was measured at 10 cm depth and the means were recorded throughout the experimental period by using a soil thermometer on a monthly basis. Second-stage juveniles (J2) in the soil samples were counted in one composite sample which was taken from 20 plots on a monthly basis during experimental set up until harvest. All composite samples were mixed and stored in plastic bags at 5 °C until nematode extraction.

The number of cysts and second stage juveniles of cereal cyst nematode and the number of root lesion nematodes in the soil samples were assessed. Six plant root samples per plot were also examined for the presence of endoparasitic nematode stages at each sampling time. Multiplication rates of the three nematodes were calculated by the ratio of the final population density in July to the initial soil population density in November. Initial population densities in March were used for the calculation of multiplication rates.

Motile stages of both *Pratylenchus* species and second-stage juveniles of *H. filipjevi* in the soil were extracted using the Whitehead tray technique (Whitehead and Hemming 1965). The final counts were expressed as numbers of nematodes per 100 g of dry soil for the different species of RLN and per g of dry soil for *H. filipjevi*. Cysts of *H. filipjevi* were also extracted, using Fenwick can method (Fenwick 1940), from 250 g soil. After cysts were crushed with a modified revolving grinding mill, the numbers of J2 were determined at minimum dilution of 30 mL and at higher dilutions as if the sample consisted of only one cyst. The viable eggs and juveniles were counted in calibrated counting chambers in 1 mL and then the number was adjusted to 100 g of soil. The reproduction factor (R) was calculated for each plot by dividing the final J2 population (Pf) density over the initial population (Pi) density in November. Numbers of J2 and eggs of *H. filipjevi* in each sample were compiled for the analysis of variance (one-way ANOVA) (SAS Institute, 1985, Cary, NC, USA).

Results & Discussion

The numbers of J2 of *H. filipjevi* extracted from soil varied for each plot during the experiments. The year round average population density of J2 was 6 J2 in 100 g of soil and root. Second stage juveniles were detected in the soil and roots throughout both growing seasons. The highest numbers of J2 were extracted from roots in April and beginning of May, 22 and 12/100 g soil, respectively; however the lowest numbers were detected in December and January (5 and 3/100 g soil), respectively . Therefore, the nematode invasion and development slowed down during November to January when the mean monthly soil temperatures were low (averaged at 3.5 °C). Then, nematode population increased when the mean temperature raised to 11.9 °C in April and May. Consequently, a strong relationship between J2 emergence and low temperatures was observed during the cold period.

The first white females on the roots were observed on May, when the soil temperature was approximately 14.1 °C and greatly increased in June. Afterwards, white females turned into light brown cysts during June and July. The greatest numbers of brown cysts (26 cysts in 250 g of dry soil) were observed in July and August of both growing seasons. These results suggested that the population of *H. filipjevi* displayed only one peak in each growing season (Figure 1).

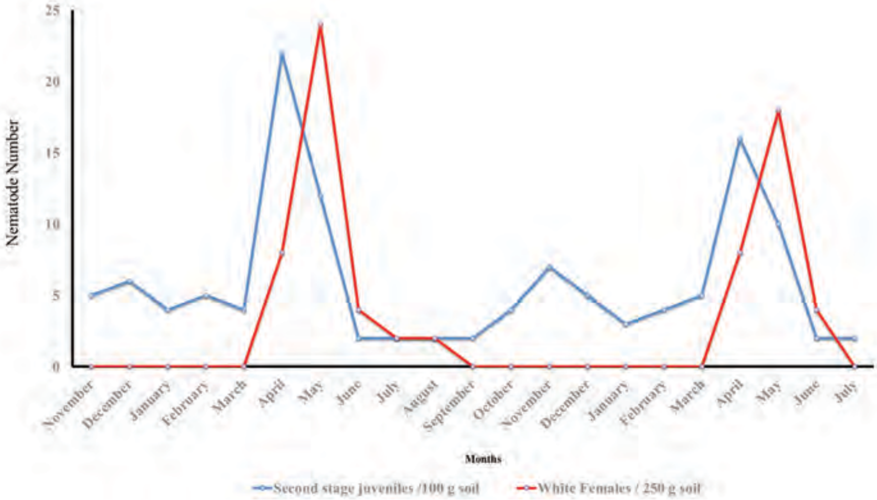

Figure 1. Population dynamics of white females and second-stage juveniles (J2) of *Heterodera filipjevi* in the soil during 2014-16 (Δ = J2/100 g soil; = White females/250 g soil).

The quantity of individual *P. thornei* extracted from root tissues varied. All stages of nematodes were detected in roots throughout both growing seasons, though the greatest numbers of nematodes were extracted from roots during May and June as *P. thornei* populations increased from early spring (March) to mid-summer (July). Nematode populations tend to decrease from the late summer (August) to the end of winter, before starting to increase again through to May (early spring). There were significantly more *P. thornei* in the lowest density plots during January and February. However, *P. thornei* had reached the highest density in plots between June and July. It was determined that there were more *P. thornei* in the plots as was in the first sampling results. Average *P. thornei* population density was 115 nematodes in 100 g soil and root, but reached up to 240 nematodes/100 g soil and root in June and July. *P. thornei* population densities indicated a positive correlation with soil temperature (R^2 = 0,9515; y = 0,362x2 - 0,9692x + 2,7773).

Population numbers of *P. neglectus* were variable and all developing forms of the nematode were observed in root tissues during the experiment. This variation in population numbers may be caused by variable soil temperatures and moisture (May through July) and availability of host plants (September through November). *P. neglectus* populations were positively correlated with temperatures between 20-25 ˚C (the average value of May); while it was negatively correlated with temperatures lower than 5 ˚C and higher than 25 ˚C. In general, nematode abundance in roots fluctuated greatly, with a distinct high during April-May in both growing seasons (coinciding with soil temperatures of 16.4-22.1˚C). Despite high nematode abundance in the soil, the population in roots was very low from December to March, which can be attributed to lower soil temperatures during these months. *P. neglectus* soil population numbers were lowest in March, at 500 and 400 nematodes per kg of soil in 2015 and 2016, respectively. Thereafter, they increased gradually until May, when there could be up to 8440 nematodes per kg of dry soil.

The results showed a positive correlation between soil temperature and density of *P. thornei* and *P. neglectus* populations. Their population density was low from November to February during the cold snow period, before increasing gradually from March to July and then rapidly decreasing between July and August. Similarly, Sahin et al. (2008) found a correlation between soil temperature and their populations in that nematode populations were low from November to March/April and increased to June/July in Ankara province of Türkiye. Nicol and Rivol (2008) reported that *P. thornei* completes its life cycle at 27 °C in 40-45 days under natural conditions. Vanstone et al. (1998) also stated that the optimal temperature for increase *P. thornei* populations was 25 °C. Moreover, Elekcioglu and Gözel (1997) also found that the numbers of *P. thornei* and *P. neglectus* in the soil was low until March and then increased during the growing season in Adana province of Türkiye.

In this study, the highest number of J2 of *H. filipjevi* in the soil was detected in April and beginning of May which is similar to the results found by Sahin et al. (2008), being the highest number of J2 of *H. filipjevi* recorded in March and beginning of April in Ankara Province in Türkiye. Hajihasani et al. (2010) reported that the hatching of *H. filipjevi* occurred during the cool period from November to March in Markazi Province of Iran. In Bolu Province, the hatching of the J2 started at a temperature of 9.7 °C and was similar to that of Ankara and Markazi populations of *H. filipjevi* in Türkiye and Iran. It was determined for the Adana's populations of *H. avenae* that hatching occurred through late autumn until the beginning of spring with the majority of juveniles hatching at 10 and 15 °C in the Mediterranean temperate climate

(Imren et al. 2012). In addition, J2 of *H. latipons* were found in the soil from November to February at soil temperature below 18 °C (Philis 1999). The variation in nematode emergences in CCN species is probably correlated with temperature of the soil in late autumn and winter. It has been demonstrated that proper soil moisture content and temperature are basic factors for hatching of J2 of *H. avenae* (Sahin et al. 2008; Hajihasani et al. 2010).

This study revealed the regional occurrence of *Heterodera filipjevi*, *Pratylenchus thornei* and *P. neglectus* in wheat growing areas of Bolu, Türkiye. The study results suggest that further studies needed on *Heterodera filipjevi*, *Pratylenchus thornei* and *P. neglectus* to develop an effective integrated control strategy to maintain nematode populations below economic damage threshold.

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Study of cereal cyst nematodes of the genus *Heterodera* in the provinces of northern Algeria behavior and tests of 4 varieties of durum wheat against this nematode

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Summary

Our work is oriented on three essential aspects; the field survey on *Heterodera* infestations, the molecular identification of the species presents in certain provinces of northern Algeria and the impact of *Heterodera avenae* populations on the yield components of four varieties of durum wheat. Nematological analysis of 54 soil samples taken from 29 communes in 14 cereal provinces revealed infestations in 92.58% of the provinces surveyed. The degree of infestation varies from region to region or even within the same region. Only Boumerdes remains not infested by nematodes. Cereal cyst nematodes were found in all three groups of surveyed areas with a frequency of occurrence of 85%. The highest density of nematode was found in semi-arid regions (Ain Defla, Bouira, Setif, Batna, Mila, Constantine, Oum Bouaghi and Tebessa) with 98.4 cysts / 100 cm^3 of soil with an average frequency of occurrence of 88.6%. These were quantified in arid provinces (Relizane and Bordj Bouariridj) with an average density of 17.7 cysts / 100cm^3 and 11.06 second stage juvenile / cm^3 of soil. This represents 75% of the infested plots. In addition, the lowest abundance of the nematode is reported in samples from the subhumid group (Souk Ahras, Algiers, Guelma) with 15.2 cysts / 100 cm^3 and frequency of occurrence of 73.33%. These densities are considered high which become more threatening for crops. The molecular identification using 20 populations of CCN sampled from cereal areas in northern Algeria showed the identification of *H. avenae, H. hordecalis, H. carotae* and *H. cruciferae*. Ten populations of cysts collected from Oum Bouaghi, Ain Melila1, Ain Melila2 and Ain Melila3, Seriana, Ain Bessem, El Yachir, Bordj Khaled, Guelma b and Merahna are identified as *H. hordecalis*. The species *H. avenae* was found in five fields in Constantine, El Asnem, Oued Smar, Hammem Sokhna1 and Hammem Sokhna2. *H. carotae, H. cruciferae* is reported for the first time in Algeria, it was found in Tadjenanet2 and Merahna, while *H. crucifera* was in Meskiana, Khmiss Meliana and Relizane. The impact of *H. avenae* isolated from Ain Defla, was carried out on four durum wheat cultivars at the ENSA horticultural station. Plant growth parameters, crop yield and nematode multiplication rate were determined. The results indicated varying sensitivity of the varieties tested, they are considered to be favourable hosts for the development of *H. avenae*. This parasite developed in a notable way on Mohamed ben Bachir and Simeto. They recorded yield losses of over 40% during the two years of the trial. We have found that Hedba and Ofanto are the most nematode tolerant varieties.

Introduction

Grains have been an integral part of human nutrition for thousands of years. They have played an important role in the development of human civilization. Cereal crops represent more than 70% of the area devoted to food crops. In 2017, wheat *(Triticum aestivum* L.) is grown on more land than any other cash crop with 218.4 million hectares. It continues to be the main source of food grains for humans (FAOSTAT 2020).

Due to the increase in world population, the world demand for wheat is estimated at 860 million t in 2025 (Marathee and Gomez-MacPherson 2001). In Algeria, cereals are grown in semi-arid areas on 3.3 million hectares:40% of the useful agricultural area. In 2017, total cereal production produced 3.5 million t, 17% below the previous five-year average (2012-2016) and 5% above 2016 (FAOSTAT 2020). Unfortunately, this figure is still insufficient to meet the demand of the growing population in Algeria. Cereal production is often confronted with various biotic and abiotic constraints. In addition to the vagaries of the weather and the calcareous nature of the soil, the poor management of crops linked to the lack of mechanization in the preparation of the soil, the lack of fertilizers and the absence of weeding, biotic factors are part of the yield reduction component. (Mokabli et al. 2002).

Among the biotic agents that limit cereal production, cyst nematodes of the genus *Heterodera* CCN, cause considerable losses to this type of crop.

In Algeria, studies carried out on cereal cyst nematodes have revealed their presence in several cereal-growing areas. However, research work relating to this parasite has been limited to its detection in large cereal-growing areas and no study has been undertaken on production losses and consequently on its economic impact on cereal yields (Rivoal and Nicol 2009; Mokabli et al. 2001).

Given their bio-ecological and agro-economic importance, we were interested in expanding investigations to other regions of the country, their diversity, their evolution and their reproduction on different varieties of wheat, in order to propose an adequate control strategy.

Our research theme on cereal nematodes in certain regions of Algeria aims to focus on the *Heterodera* group. Based on existing databases, we studied the infestations of the plots to make an assessment of the seriousness of the situation. We have tried to complete the work carried out by different authors on the biological and biochemical characterizations of the different species present in Algeria. Finally, the study of the impact of nematode densities on yield components is essential to quantify the losses estimated on the basis of tests of the nematode in question alone and without any associated pathogen.

Methods

Cyst extraction

The survey was carried out during the summer period, after the cereal harvest in 2017-18 and 2018-19. The choice of our study sites focused on 54 plots producing cereals (wheat, barley and oats) spread over 14 wilayas of the North, Center, East and West

The extraction of *Heterodera* spp. cysts was carried out at the nematology laboratory of the higher national school of agriculture (ENSA), Algiers by the Fenwick (1940)

Larvae extraction from soil

From each sample, 100 cm^3 of soil were used to extract the vermiform stages of *Heterodera* spp. The soil samples from each plot are placed on 40 µm sieves, themselves placed in containers (large Petri dishes) containing tap water. The nematodes by rehydrating become active and cross the meshes of the sieves. We kept the samples in extraction for 2 days, checking that the samples always remained moist and did not dry out with evaporation. After pouring the suspensions containing the nematodes into graduated beakers, adding water up to 200 µl, we left the samples to stand overnight. The larvae descend to the bottom of the beaker, which allowed us to lower the water level to 100 ml. Using a pipette, the solution was homogenized and 5 µm was taken for counting the larvae using an optical microscope.

The evaluation of the infestation

For each plot studied, the population density (P.D.) of nematodes per 100 cm3 of soil, the frequency of occurrence % (F.O. %) and the prominence value (P.V.) are calculated according to (Norton 1978) as follows: P.D = average number of nematodes per 100 cm3 of soil. F.O.% =s (number of samples containing nematode / total number of samples collected) * 100. P.V = population density / frequency of occurrence (Boag 1993; Baklawa 2013)

DNA extraction

The cysts were crushed and the juveniles released into 10 µl of distilled water. The juveniles were homogenized by a micro-homogenizer, then the whole lysate was placed in a 1.5 ml Eppendorf tube, 10 µl 1xPCR reaction mix [75 mM Tris-HCl (pH 8.8), 20 mM (NH4) 2SO4, Tween 20

0.01% (v/v)] and 2 µl proteinase K (600 µg/ml; Qiagen GmbH, Hilden, Germany) are added to the lysate. The tube was incubated at 60°C for 30 minutes, then 5 minutes at 95°C to remove proteinase K. The tube was centrifuged at 16,000 rpm for one minute. The supernatant was carefully removed without disturbing the pellet, and transferred to another Eppendorf tube and stored at -20°C until further use. The molecular identification was carried out by the PCR method, with primers F194 and F195 (described by Ferris et al. (1994) to amplify the ITS regions, including the ITS1, ITS2 and 5.8S ribosomal gene, as well as parts of the 18S and 28S ribosomal genes using a T100 thermocycler (Bio-Rad, California, USA).

Phylogenetic analyzes

ITS sequences were used to explore the intra-specific genomic variability of *Heterodera* species and to determine the phylogenetic relationships between collected populations and representative populations of *Heterodera* from different countries available in the GenBank database. A total of 24 nucleotide sequences are involved in the phylogenetic analysis performed with MEGA7 (Kumar et al. 2016). Evolutionary history was inferred using the neighborhood method (Saitou and Nei 1987), based on evolutionary distances calculated using the Tamura-Nei method (Tamura and Nei 1993). Bootstrap support was calculated for all analyzes using 1000 replicates (Felsenstein 1985)

Varietal behavior

We conducted a test on the behavior of four varieties of durum wheat (*Triticum durum*) against a population of *Heterodera avenae* originating from Ain Defla. to assess the relationship between the population density of *H. avenae,* the growth and yield of the host plant, as well as the reproductive capacity of these nematodes on the different cultivars.

The test was carried out at ENSA experimental station according to a device in blocks in randomization with 4 repetitions per variety, we choose varieties preferred by farmers. The soil used in the test was sterilized in an oven at 120°C for 2 hours, once cooled, it was sieved, then mixed with the soil (1/3 soil + 2/3 soil) to form a mixture of 1 kg per plot (25 cm in diameter, 15 cm long). Each plot received an inoculum of 20 full cysts, bagged in a small 250-micron tulle bag allowing the larvae to exit, placed at a depth of about 3 cm. Each plot was perforated to allow good drainage of irrigation water. Sowing was carried out three days after inoculation at the rate of 5 germinated caryopsis per pot. Temperatures and humidity were controlled during vegetation and the plants are fertilized and even Manual weeding was carried out each time weeds appear in the pots.

At the end of the test, the growth and yield parameters are estimated (spike length, stem height, number of spike/plants, Number of grains/spikes, weight of 1000 grains; yield was calculated by the following formula:

Yield = Number of grains / plots reported to one hectare based on the surface of the pot.

Referring to Namouchi-kachouri et al. (2006), the yield loss (P.R) is calculated by the following formula:

$$P.R = 100 - \%\ yield$$

Statistical analysis

The data collected on the population densities of cereal cyst nematodes of the genus *Heterodera* in the surveyed plots were the subject of the ANOVA analysis of variance in order to test the significance between the tested means. All data are analyzed using STATISTICA software (Version 6).

To know the influence of different factors such as: previous crop, climate, geographical origin, on the distribution of *Heterodera* spp. in the surveyed regions, we carried out a one-factor ANOVA analysis of variance (Region, Previous Cultural, Bioclimatic stage). The groupings according to the means were carried out using the Fisher test (LSD) with a confidence interval at 95% ($p<0.05$).

Hierarchical Ascending Classification of Variables (HAC): was used to identify plateaus where many classes are generated at a close aggregation distance using STATISTICA software (Version 6).

The data collected relating to the sensitivities of durum wheat varieties, against the cyst nematodes *Heterodera avenae*: Growth parameters, yield and its components, were subjected to a one-factor ANOVA analysis of variance: Varieties, by software: IBM SPSS STATISTICS 20. They were processed by Dunnett's test to identify homogeneous and non-homogeneous groups.

After calculating the rate of reproduction of the nematodes of each variety, we used a scale established by Valocka et al. (1994), to evaluate the sensitivity of the varieties, and confirm the previous results.

Results & Discussion

Our field surveys, the study of the state of infestation of the plots of some cereal regions by cereal cyst nematodes of the genus *Heterodera* showed that, except for the region of Boumerdes, all the surveyed areas are infested. This represents a rate of 92.58. Infestation levels vary from region to region and even within the same region. We mentioned that the prospected plots of Tebessa are infested with 0.8 cysts and 2 L_2 per 100 cm^3 of soil (F.O = 33.33%), going as far as Oum Bouaghi where we record 28.9 cysts with 72.25 L_2 per 100 cm^3 where F.O =70%. We reported total infestation rates in Relizane, Ain Defla, Guelma, Mila and Bouira (F.O = 100%).

This wide fluctuation of infestations is the consequence of inadequate cultural practices which allow the reproduction of this pest (rotation system, abundance of unploughed fallow land, absence of weeding, varieties used, dissemination by the wind, tillage tools, polyculture, etc.). (Labdelli et al. 2017; Righi et al. 2014).

Analysis of variance shows very highly significant and significant differences between nematode population densities expressed as number of cysts/100 cm^3 of soil and number of larvae/cm^3 of soil. They are based on the regions studied and their bioclimatic levels. The results obtained confirm that fluctuations in infestation levels are closely linked to the interaction of many factors such as climate (Smiley and Nicol 2009), rotation system (Al-Hazmi et al. 2015), cultivated varieties, soil type (Smiley and Nicol 2009), which influence the populations of the *Heterodera* spp.

Table 1. Population density (PD), occurrence frequency (FO) and prominent value (PV) of prospected regions in Algeria

Province	Samples taken	N° Infested samples		Cysts/100 cm^3 of soil	Larvae /100cm^3 of soil
Relizane	2	2	PD	4,9	12,25
			FO	100	100
			PV	4,9	12,25
Boumerdes	2	0	PD	0	5
			FO	0	50
			PV	0	3,54
Bord Bou	2	1	PD	12,8	32
			FO	50	50
			PV	9,05	22,63
Ain Defla	5	5	PD	22,9	57,25
			FO	100	100
			PV	22,9	57,25

Bouira	4	4	PD	15	37,5
			FO	100	100
			PV	15	37,5
Guelma	3	3	PD	6,4	16
			FO	100	100
			PV	6,4	16
Oum bouaghi	10	7	PD	28,9	72,25
			FO	70	70
			PV	24,18	60,49
Souk Ahras	7	5	PD	7	17,5
			FO	71,43	71,43
			PV	5,92	27,29
Batna	4	3	PD	5,7	14,25
			FO	75	75
			PV	4,94	12,34
Constantine	3	2	PD	13,8	34,5
			FO	66,67	66,67
			PV	20,47	28,17
Sétif	3	2	PD	5	12,5
			FO	66,67	66,67
			PV	4,08	10,21
Tebessa	3	1	PD	0,8	2
			FO	33,33	33,33
			PV	0,46	1,15
Mila	3	3	PD	6,3	15,75
			FO	100	100
			PV	6,3	15,75
Alger	3	1	PD	1,8	4,5
			FO	33,33	33,33
			PV	1,04	2,6

PD: Population density; **FO**: Occurrence frequency; **PV:** Prominent value

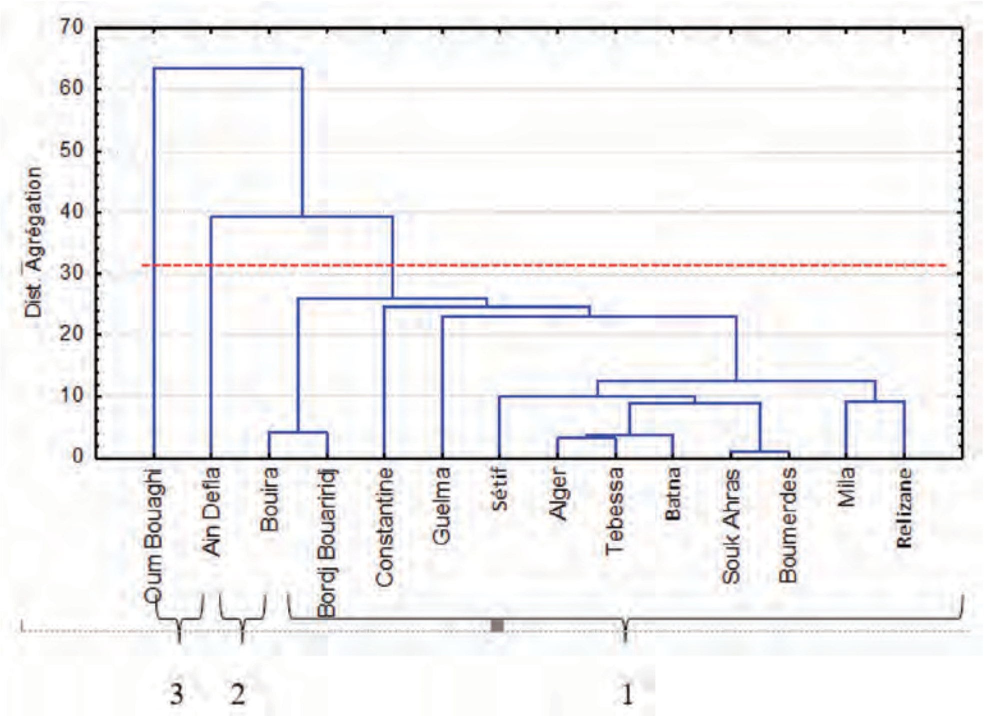

Figure 1. Hierarchical classification or prospective regions based on the level of infestation by cereal cyst nematodes.

Regarding the mastery of knowledge and the molecular characterization of the *Heterodera* group, the molecular identification of some populations of nematodes has provided new information on the distribution and frequency of occurrence of *Heterodera avenae*, *H. hordecalis, H. carotae* and *H. cruciferae* in the main wheat-growing areas in northern Algeria. It appears that *H. avenae* is more widespread compared to other species. However, the species *H. latipons* is considered absent in all the plots surveyed.

The test carried out on the impact of the nematode in question without any interaction with other pathogenic agents on four varieties of durum wheat (*Triticum Durum*) against a population of *Heterodera avenae* from Ain Defla carried out over two consecutive years is carried out at the ENSA horticultural station. Finally, the goal is to assess the effect of this nematode on the growth and yield of the durum wheat varieties tested. We showed that *Heterodera avenae* is a pathogenic agent harmful to the culture of durum wheat in Algeria more particularly to the cultivars Mohamed ben Bachir and Simeto.

The other varieties tested also proved to be favorable hosts for the development of the nematode *Heterodera avenae* in a less aggressive manner; these are Hedba3 and Ofanto. Yield loss estimates are generally plotted against plot area and reported as a unit area of one hectare. They reveal that under controlled conditions, nematode populations alone can reduce yields by more than five quintals.

Table 2. Result of identification of some nematode populations of the genus *Heterodera* in some infested regions, with their accession numbers

No	Provinces	Area	Species	Accession Number
1	Oum Bouaghi	Oum Bouaghi	*H. hordecalis*	MK848400
2	Oum Bouaghi	Ain Melila 1	*H. hordecalis*	MK840641
3	Oum Bouaghi	Ain Melila 2	*H. hordecalis*	MK848401
4	Oum Bouaghi	Ain Melila 3	*H. hordecalis*	MK840638
5	Oum Bouaghi	Meskiana	*H. crucifera*	MK848395
6	Batna	Seriana	*H. hordecalis*	MK840640
7	Constantine	Constantine a	*H. aveane*	MK848396
8	Bouira	Ain Bessem	*H. hordecalis*	MK848399
9	Bouira	El Asnem	*H. aveane*	MK840645
10	Bourdj Bou Ariridj	El Yachir	*H. hordecalis*	MK840642
11	Ain Defla	Khmiss Meliana	*H. crucifera*	MK848393
12	Ain Defla	Bordj Khaled	*H. hordecalis*	MK848402
13	Guelma	Guelma	*H. hordecalis*	MK840637
14	Mila	Tadjenanet 2	*H. carotae*	MK840644
15	Relizane	Relizane	*H. crucifera*	MK848394
16	Souk Ahras	Merahna	*H. hordecalis*	MK840639
17	Souk Ahras	Merahna	*H. carotae*	MK840643
18	Alger	Oued Smar	*H. aveane*	MK840646
19	Setif	Hammem Sokhna 1	*H. aveane*	MK848397
20	Setif	Hammem Sokhna 2	*H. aveane*	MK848398

Table 3. Growth parameters and yields of varieties tested

Variety	Year		Spike lenght	Stem height	Spike/ plant	Grains/ cob	PMG	Grains/ plot
Hedba3	**1st**	**W**	7.25±1.708^{c}	71.75± 16.820^{b}	2.50±0.577^{ab}	26.00±5.292^{a}	37.75±9.287^{a}	327.50±104.921^{ab}
		I	5.50±1.000^{b}	55.50±11.030^{b}	1.25±0.500^{a}	24.00±5.228^{a}	31.75±7.676^{a}	156.25±91.413^{a}
	2nd	**W**	8.2500±0.95743^{b}	52.2500±4.78714^{a}	1.7500±0.50000^{a}	26.7500±2.75379^{a}	35.5000±4.50925^{ab}	230.0000±55.97619^{a}
		I	7.0000±0.00000^{c}	49.5000±9.46925^{a}	1.5000±0.57735^{a}	24.0000±5.22813^{a}	31.0000±2.94392^{ab}	180.0000±82.05689^{a}
MB	**1st**	**W**	6.00±0.816^{bc}	63.50±12.234^{b}	2.75± 0.957^{ab}	25.50±3.416^{a}	34.25±6.946^{a}	353.75±136.221^{ab}
		I	4.50±0.577^{ab}	53.25±5.058^{b}	1.75±0.500^{ab}	23.75±3.594^{a}	32.00±5.944^{a}	208.75±71.923^{ab}
	2nd	**W**	4.7500±0.95743a	55.5000±8.34666^{a}	3.2500±1.70783a	26.0000±3.16228^{a}	33.2500±6.39661^{a}	442.5000±263.77073^{a}
		I	3.2500±0.50000^{a}	53.2500±10.30776^{a}	1.7500±0.50000^{a}	24.5000±4.04145^{a}	23.0000±16.26858^{a}	215.0000±75.05553^{a}
Ofano	**1st**	**W**	4.25±0.5^{a}	41.25±3.500^{a}	2.00±0.816^{a}	24.50±1.291^{a}	34.50±6.028^{a}	243.75±96.728^{a}
		I	4.00±0.000^{a}	38.00±4.082^{a}	1.75±0.500^{ab}	22.25±4.787^{a}	33.50±4.359^{a}	200.00±82.865^{ab}
	2nd	**W**	4.5000±1.29099a	54.0000±6.87992^{a}	3.0000±0.81650^{a}	24.5000±2.51661^{a}	31.7500±5.73730^{a}	372.5000±135.00000^{a}
		I	4.5000±1.29099^{b}	50.2500±5.31507^{a}	1.7500±0.95743^{a}	20.5000±1.29099^{a}	31.0000±2.70801^{ab}	181.2500±107.42245^{a}

Semeto	1st	**W**	5.25±0.5^{ab}	44.50±2.082^{a}	3.50±0.577^{b}	28.75±2.986^{a}	42.75±4.573^{a}	505.00±113.578^{b}
		I	4.00±0.816^{a}	40.50±4.796^{a}	2.25±0.500^{b}	26.25±2.500^{a}	36.25±5.560^{a}	298.75±92.410^{b}
	2nd	**W**	5.2500±0.95743^{a}	45.5000±4.65475^{a}	3.0000±0.81650^{a}	28.5000±1.73205^{a}	42.7500±2.87228^{b}	426.2500±109.34464^{a}
		I	5.0000±0.00000^{b}	43.5000±4.72582^{a}	1.7500±0.50000^{a}	26.0000±1.41421^{a}	37.5000±4.79583^{b}	227.5000±66.52067^{a}

W: Witness; **I**: infested; **PMG**: thousand grain weight

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Session II

Climate changes impacting the importance and population dynamics of cereal nematodes on wheat

Biotic and abiotic constraints and the future of integrated root-health management in semi-arid wheat production

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Introduction

Food production and food security are now being severely impacted by biotic and abiotic constraints on a global scale. The importance of both factors in reducing crop yield has increased significantly and will influence how agriculture is conducted in the future and how we feed the world's poor and hungry. These constraints also influence how crop health will be addressed in the future. In the present highly complex social, political, agricultural and ecological environment the topic of root-health management will play an important role in food production and is the topic of this paper.

We are running out of time globally with regards to improving yield to feed the masses as influenced by shifts in climate. Central and West Asia and North Africa (CWANA) is a climate hotspot and one of agricultures' most water-stressed ecological zones. It is the region where constraints such as climate variability are having a major limiting impact on rainfed agroecosystems, food production, food security and human livelihoods.

Over one third of the earth's land surface is arid or semi-arid, receiving less than 400 mm annual precipitation and supporting over 20% of the world's population. The populations in these regions are expanding at a rate which current agricultural practices cannot adequately support with food using present production systems. Countries in this zone are experiencing prolonged periods of elevated temperatures and suffering through drought conditions that are affecting both yield and human wellbeing. The demand for wheat will double in the next 30 years as populations increase. Therefore, there is an urgent need for more research and development to improve wheat productivity regionally and globally.

Wheat production in the semi-arid zones around the world is extremely important and especially true in the countries of CWANA. In Central and West Asia and North Africa, agriculture is characterized by large variations in agro-ecology, farming systems, moisture, temperature, soil types and cultural practices and therefore is hyper-sensitive to biotic and abiotic constraints. Food production in many semi-arid zones is closely related to interrelationships involving the above factors but also to: pest and disease pressure, high post-harvest losses, degraded soils, poor quality seed, lack of fertilizer, non-existent integrated pest management, and importantly a lack of credit for inputs.

Drivers and constraints

Human population growth, energy and climate changes are *drivers* of change that cannot be significantly influenced by humankind. These drivers have a great impact on how agricultural production is conducted and of course on ultimate yields attained. Human population growth continues rise with densities in sub-

Saharan Africa alone projected to double in the next 30 years. CWANA has the second highest population growth rates globally, with some countries growing at 3.5% per year. Recent, unpredictable shifts in climate are responsible for serious crop loss due to farmer's inability of react.

Constraints, both biotic and abiotic, are limiting factors that can be manipulated by humans to improve food production. The major constraints to production include: pests and pathogens, land degradation, soil fertility, water availability, land ownership, and global and regional markets just to name a few.

Drivers and constraints will have a significant influence on the future of food production and food security. The manner in which we manage agricultural production in the future to offset these limiting factors was discussed in detail in a recent open-access book: *Transforming Agriculture in Southern Africa* (Sikora et al*.,* 2020).

Farm size, food production and root health management

Yields in the semi-arid production zones tend to be lower than in other climatic regions because of a number of known agro-ecological limiting factors. In some semi-arid countries, for example, farm size limits production. An estimated 98 percent of the farmers in the world grow crops on less than 2 ha. In most of Africa, India and China farm size is around 1.2 ha and decreasing. Resource limited farmers in these situations often have little to no access to quality seed, IPM, fertilizer, extension services, markets, nor credit. The yield of these farmers is also greatly affected by a lack of knowledge of root-health problems and by their inability to use modern technologies available to larger farmers.

Climate change and climate variability

These two interrelated elements are now becoming the most important factors limiting food production (Sikora et al*.,* 2020, 2022). This impact can be seen in the recent: droughts, extensive forest fires, high temperatures, human conflicts, and flooding. Long-term climate change and short-term climate variability are and will continue to have massive impacts on agriculture in CWANA and other semi-arid regions of the world.

Climate change refers to multi-decadal temperature and precipitation trends associated with anthropogenic warming.

Climate variability refers to interannual and seasonal changes in weather patterns locally or regionally.

There is a need to redesign how crops are grown and how pest and plant diseases are managed to offset the impact of these two drivers. This is especially true with regards to root-health as will be discussed later. Rethinking how crops are grown in the semi-arid zone will be a major objective of agricultural science research in the near future.

Water availability

Available water for crop production in most semi-arid zones including CWANA is becoming scarcer as seen in drastically sinking water tables. In the future the lack of available water for plant growth will affect all crops in CWANA as well as global food production in other countries with semi-arid climates. Therefore, there is a need to change the way crop production systems are designed with regards to water use. Water availability is rapidly declining worldwide not only due to agriculture, but also due to demand from non-agricultural sectors as well as use for environmental protection purposes.

Root-health management

Sustainable improvements in crop production can be achieved by maximizing water use efficiency as opposed to just increasing yield per hectare through management of farm resources. An extremely important aspect of crop production that is almost always overlooked when attempting to improve water use efficiency is the presence of nematodes and fungal pathogens impacting root-health. Root-health management is not only about breeding for faster root growth, heat resistance or climate-smart cultivars. Breeding has to include resistance to nematode and/or fungal pathogens otherwise inadequate root growth and depth penetration will not support a healthy crop or produce good yields. Root-health management has to be designed to improve root-health to the major constraints both biotic and abiotic in the semiarid regions.

Nematodes and pathogens on wheat

Root-health in CWANA and all other semi-arid regions of the world is negatively impacted by a wide range of both cyst and lesion nematodes as well as important fungal pathogens. The importance of different species of nematodes and pathogens will be covered by other speakers at this meeting and not covered here. Both groups of pests have major root damaging importance on wheat and other crops in the semi-arid regions of CWANA. However, whereas fungal pathogens can often be managed with fungicide seed treatment, the use of nematicide seed treatment for nematode management in cereal crops is a technology of the future. In some semi-arid countries, for example in most of Africa, fungicide treatment of seed is not practiced.

Complex nematodes-pathogen interactions

It is well known that plant parasitic nematodes and soil borne fungal pathogens, both alone and in combination, cause severe damage to the root system. In some cases nematodes have been shown to break plant resistance to fungal pathogens and in other instances fungal infection increases damage from nematodes. Resource limited farmers around the world are not aware of root-health problems caused by nematodes and fungal pathogens due to the fact that seed treatments are not always used or available . Fungicide seed treatments will prevent most pathogen infections and limit complex interactions. However to date, problems with nematode early root penetration root stunting and subsequent root deformation cannot be managed with nematicide seed treatments.

Root architecture and root health

Soil degradation and low levels of organic matter in semi-arid soils negatively affects plant health by reducing nutrient availability and water and nutrient retention. These two constraints also influence root architecture and ultimately yield. In addition, the lack of organic matter reduces biotic suppressive activity in soil by limiting eco-system services that regulate soil-borne nematode and disease.

However, extremely important for yield under water stress conditions, is the direct impact of nematode and fungal pathogens on root system architecture. Nematodes and pathogens damage root tips causing a flattening and stunting of the root system thereby reducing root depth penetration. In addition, root lesion nematodes and root-rotting fungi, alone and in combination, weaken root health further by degrading overall root tissue which inhibits nutrient and water uptake.

Future options for improve root-health and yield

The following points need to be considered in the immediate future in order to offset the impact of the constraints mentioned above on root-health and yield in the semi-arid production zone in both CWANA as well as globally:

- Climate resilient production systems are urgently needed in CWANA.
- Climate-smart research must take a holistic approach to solve abiotic/biotic constraints.
- Abiotic constraints require improved root architecture and healthier root systems.
- Nematode and pathogen destruction of root architecture needs is extremely important for plant growth.
- Breeding for root-health is a *best-bet* approach to deal with abiotic constraints affecting plant growth.
- Breeding for heat and water stress without including root-health is counter productive.
- Climate resistant cultivars need to have proven root-health properties.
- Optimizing crop health requires a *vertical instead of horizontal* approach to management and breeding.
- Systemic seed treatments with nematicide/fungicide active compounds will improve root-health.
- Farmers need additional training in integrated pest management as it relates to root-health.
- New breeding technologies like CRISPER may have great impact on soil-borne nematodes and pathogens.
- Nematode and pathogens require long-term research and improved funding.
- The CG needs to increase research support on root-health integrated management for all major food crops.
- Urgently required is a One-CGIAR program that supports breeding for resistance to soil-borne pest, pathogen and abiotic constraints for improved on root-health.

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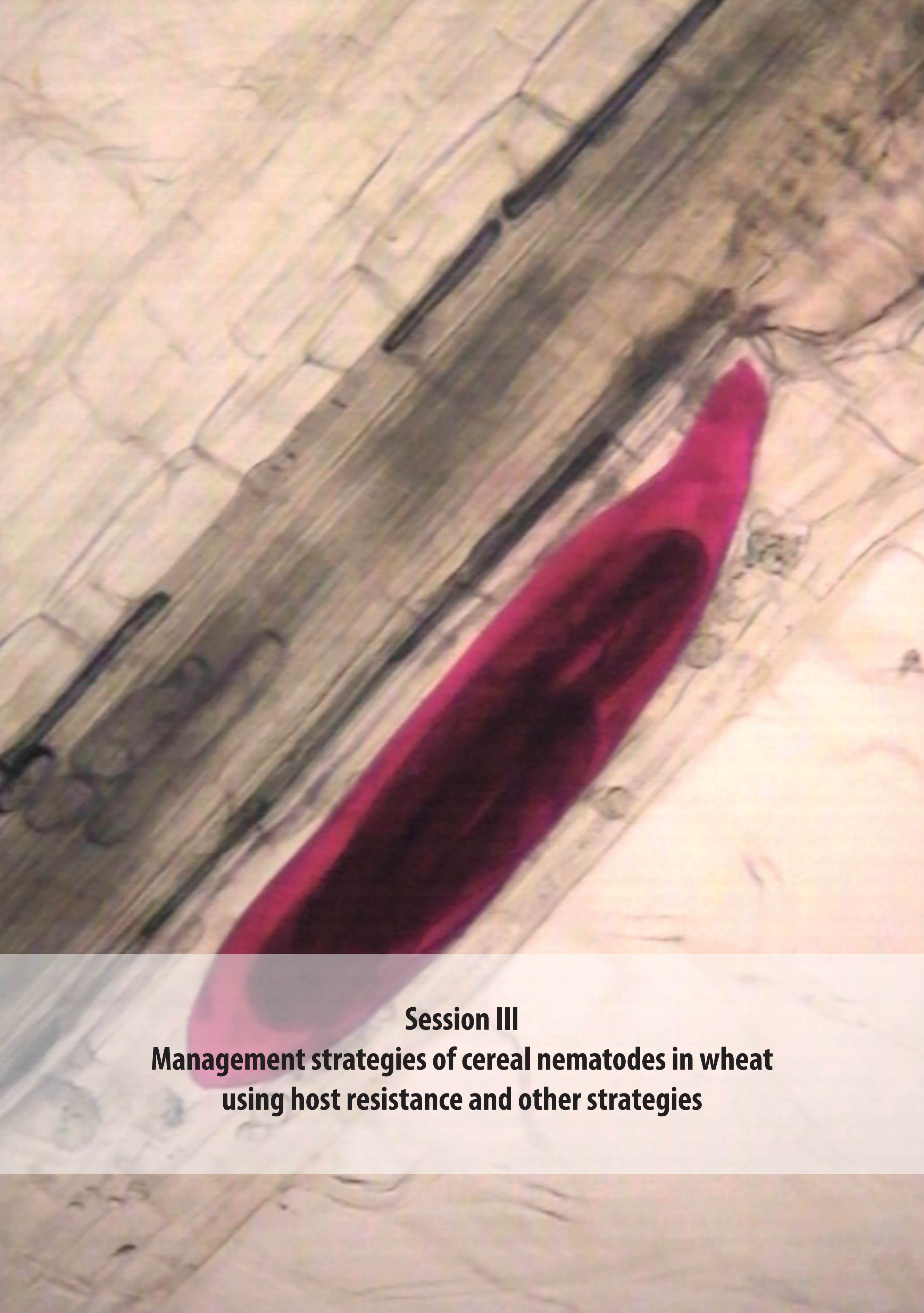

Session III

Management strategies of cereal nematodes in wheat using host resistance and other strategies

Cyst nematodes are a hot-spot for the discovery of new nematode antagonistic fungi with potential to be developed into nematode biocontrol agents

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Historic background of nematode parasitic fungi

Nematode parasitic fungi have a long history in mycology: In 1877 Julius Kühn described *Catenaria auxiliaris* as a nematophagous fungus from the sugar beet cyst nematode (*Heterodera schachtii*) and suggested that such fungi might play a beneficial role in agriculture (Kühn 1877). Since then many fungi have been studied that are feeding on or parasitising nematodes: Examples are the nematode-trapping fungi that actively capture free living nematodes with highly sophisticated hyphal structures (Zopf 1888) and fungi that parasitise the eggs of sedentary plant parasitic nematodes such as cyst forming or root-knot nematodes (Nordbring-Hertz et al. 2011).

Nematophagous fungi have been reported to suppress nematode populations in different cropping systems across the world. In the 1980s the concept of suppressive soils was established based on the observation that some agricultural soils under intensive cropping were less conducive to nematode infections of the crops. It was reasoned that this could be due to the build-up of antagonistic fungal and bacterial populations (Kerry 1988). As an example, surveys on *Heterodera avenae* under continuous cereal cropping systems revealed that a low nematode population density in soil was correlated with high abundance of egg-parasitic fungi (Kerry and Crump 1998). Many fungal species have been isolated from nematode cysts, however only few links could be made between individual fungal species or consortia thereof that could be the causal agents of that observed 'natural nematode control' (Lopez-Llorca et al. 2008).

Recent discoveries of nematode egg-pararasitic fungi and their biology

In recent years, Ashrafi et al. (2017a) developed a new isolation technique that allowed the specific isolation of parasitic fungi from individual nematode eggs. This resulted in the finding of several fungal species obtained from cysts of *Heterodera filipjevi* collected in Turkey (Ashrafi et al. 2017a; Ashrafi et al. 2018; Ashrafi et al. 2017b). These species were all isolated from fungus-infested eggs of cyst nematodes collected in 2013 from fields naturally infested with nematodes, where a sharp reduction on nematode populations was observed during two consecutive growing seasons. Thus, all of these species could also play a significant role in the natural reduction of plant parasitic nematodes in agroecosystems. In addition, all strains that had been isolated by the new technique have fulfilled Koch's postulates using cysts and eggs of *H. filipjevi* and thus their parasitic nature against cyst nematodes has been confirmed causally *in vitro* (Figure 1). The same approach was applied to isolate egg-parasitic fungi from the sugar beet cyst nematode *H. schachtii* collected from naturally nematode-infested fields in Germany. The fungal isolates obtained, also fulfilled Koch's postulates, and showed suppressive effects on nematode population densities in greenhouse experiments (Nuaima et al. 2020). Given the fact that chemical control methods previously used in the control of plant parasitic nematodes are in the meantime banned in the EU and many other countries, biological control methods to directly reduce the nematode numbers in agricultural soils, are highly sought for.

Molecular phylogenetic analyses of the fungal strains revealed that most of these antagonistic fungi isolated from nematode eggs represented so-far undescribed species, which we described as new taxa, accordingly, (Ashrafi et al. 2017a; 2017b; 2018) or which are currently in the process of being described. So far, all isolated fungal strains belonged to three orders of Ascomycota, *i.e.* Helotiales, Pleosporales and Hypocreales. Furthermore, we found several identical or near-identical DNA sequences deposited in GenBank, which stemmed from endophytic fungi isolated from the roots of wheat, barley and wild grasses, but also from Brassicaceae and different shrub and tree species. This finding suggested that the fungal strains that had been isolated by us as parasites from nematode eggs might also live during a part of their life cycle or alternatively as root endophytes of a variety of plant species including agronomically relevant crops like wheat and barley. To test whether the strains isolated from nematode eggs have the capacity to grow endophytically, a strain of a new pleosporalean species (under species description) was exemplarily tested for its ability to colonise the roots of wheat. The light microscopic studies (bright field and laser scanning microscopy) showed that the fungi could enter the wheat root and grow intercellularly but also enter cortical and even endodermal cells through specialised structures and thus have a more intimate, intracellular interaction with the plant cells (Figure 2). In these preliminary studies no negative effects or symptoms could be detected on the wheat plants, thus fulfilling the classic definition of an endophyte.

The path from discovery to application

Because of the discovery of a significant number of so-far unknown egg parasites of cyst nematodes using the new isolation technique in a small geographic setting and with two hosts only we assume that the isolation technique will continue to provide a constant supply of egg-parasitic fungi isolated from various species of cyst nematodes in different geographic regions. All these newly discovered egg-parasitic fungal strains can be regarded as potential future biocontrol strains. To move closer to application of these strains in agricultural practice several aspects have to be evaluated, optimised and established. Thus, a research project was initiated that includes several research groups with special expertise in: 1) isolating and characterising of the strains and their control potential, 2) evaluating the capacity of the fungal strains to produce natural compounds and elucidating the chemical structure of these compounds and their bio-activity, 3) fermenting and formulating of strains, and 4) a company with experience in scaling-up and mass production of fungal strains for use in organic agriculture, respectively. The overarching main aim of the research consortium is to get closer to application of these strains in agricultural practice and identify opportunities and constraints. The results obtained in the project so far will be presented.

In project part (1) several new fungal species have been characterised, and it was shown that fungi obtained from eggs of *Heterodera filipjevi* could also infect *H. schachtii*. Also, an in vitro population of *H. schachtii* was successfully established by multiplication of nematodes on oilseed radish (*Raphanus sativus*) to continuously produce healthy cysts of *H. schachtii*, which can be used in further infection experiments.

After the general proof of parasitism of the isolated strains by fulfilling Koch's postulates *in vitro*, the control efficiency of the strains is tested in a close-to nature complex tripartite system consisting of host plant, cyst nematode and nematode-antagonistic fungus under controlled conditions in the green house. By doing so the different control efficiencies of the various strains are to be evaluated to pre-select the strains with the highest control effect. First results are available for this step and the system is being optimised to allow robust and repeatable testing of the control capacities of the strains under greenhouse conditions.

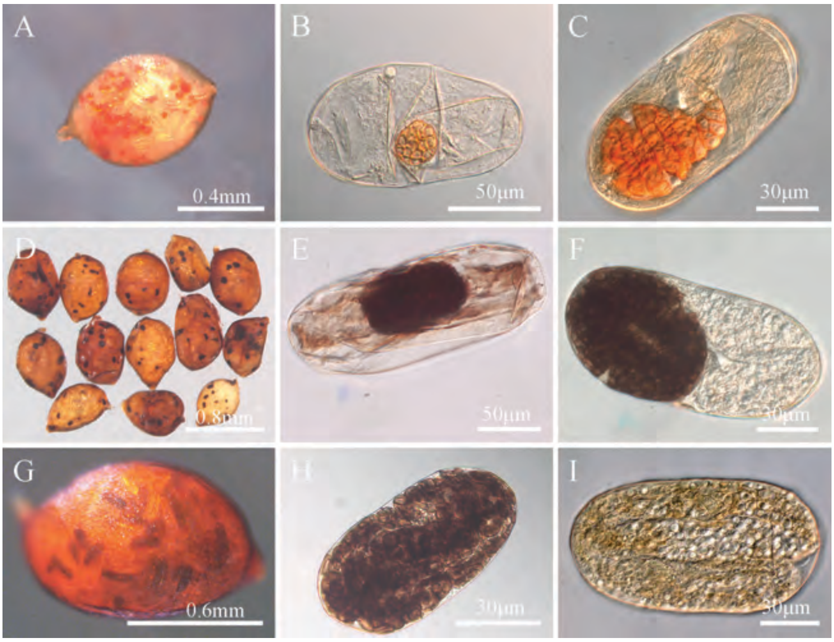

Figure 1. Recently described fungi isolated from eggs of *Heterodera filipjevi* collected from naturally infested fields. **A, C**) Nematodes infected by Ijuhya vitellina, **A, B)** Cysts and eggs of H. filipjevi naturally infested with I. vitellina. Infested eggs accommodate one, occasionally two globose microsclerotia. **C)** In vitro colonisation of a nematode egg by hyphae developed into a microsclerotium. **D – F)** nematodes infected by Niesslia (= Monocillium) gamsii, **D, E)** Cysts and eggs of H. filipjevi naturally infected by N. gamsii. Infested eggs contain one or two microsclerotia. **F)** Formation of microsclerotia by development of fungus inside the nematode body cavity. **G – I)** Nematodes infected by Polyphilus sieberi. **G)** A Nematode cyst of H. filipjevi infected in vitro, **H)** naturally infected egg. Infected eggs were colonised by moniliform and strongly melanised hyphae. **I)** fungal colonisation of a developing juvenile in vitro.

In project part (2) the fungal strains are screened for secondary metabolites and the compounds isolated are structurally elucidated. These comprised for example chaetoglobosins A and B, cyclodepsipeptides such as arthrichitin B and D, and the new cyclic lipodepsipeptide ophiotine (Ashrafi et al. 2017a; Helaly et al. 2018; Moussa et al. 2020). Some of these compounds showed nematicidal activities and thus could play an important role in the observed nematode-parasitic nature of these fungi. In addition, testing the substances for potential cytotoxicity against human cell lines is important for risk assessment of strains that will potentially be applied in larger quantities in the field. In part (3) of the project the growth parameters of selected strains are optimised to obtain maximum yield comparing solid state and liquid fermentation. The production of the strains must be suitable for the formulation process that is following the fermentation, and this must be adapted to the envisaged application of the strains to the fields. The currently best performing strains were shared with the collaborators of the company responsible for part (4) of the project who are currently trying to further optimise and adapt the growth parameters to larger volume fermenters.

Integrating the different expertise of the collaborating partners has allowed for significant progress, by the application of different approaches to the same topic from various angles. It also highlighted where the greatest methodological problems still exist to come closer to the application of fungal strains as biocontrol agents against cyst nematodes in agriculture. By addressing and solving these problems it is expected that a robust pipe line will be available in the near future enabling data-based decisions on each of the steps from the discovery of new antagonistic strains to choosing and applying the best of these strains in a suitable formulation and application technique.

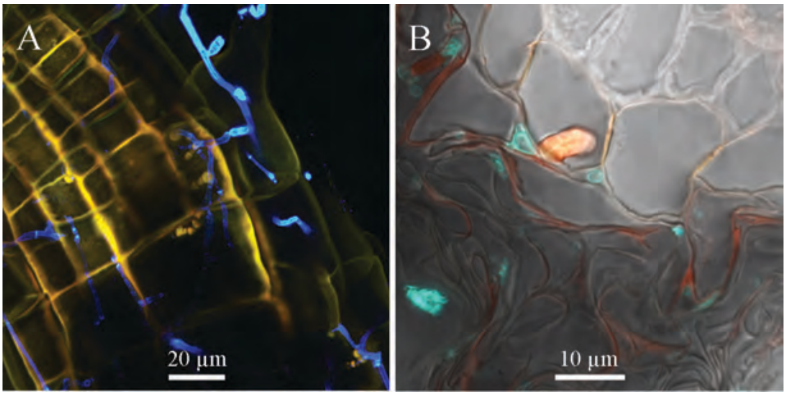

Figure 2. Confocal laser scanning micrographs of the pleosporalean strain YE1 colonising of wheat roots. **A**) Development of fungus inside the system; **B**) cross section of root cells displaying development of the fungus inside the root cell by formation of a papilla structure.

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Resistance reaction of some bread and durum wheat cultivars to the seed gall nematode, *Anguina tritici* (Steinbuch, 1799) under field conditions

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Summary

The effects of the seed gall nematode *Anguina tritici* on agronomic characteristic of 25 bread and 5 durum wheat were investigated at the Trakya Agricultural Research Institute, Türkiye, during 2015-2016 growing seasons. The study was carried out to evaluate the reaction of wheat cultivars to the seed gall nematode under the field conditions. The obtained results showed a wide range of variation among the wheat cultivars in terms of infection grain ratio. The highest infected grain ratio was found in the bread wheat cultivar, Kenanbey with 71.4% of kernel infection and Zühre, the durum wheat by 24.9%, whilst the lowest was revealed in wheat cultivar Karahan with only 0.2% infected grain. Karahan was also tested against other plant parasitic nematodes such as *Heterodera* species and was ranked as moderately resistant. This study is important to the breeding programs so local susceptible cultivars can be replaced with high yielding and resistant one.

Introduction

Wheat is main crop for humanity with its 220 million hectares cultivated area, 767 million tons of grain yield of which 184 million tons are subjected to international trade (Anonymous 2021a). The world population is expected to increase exponentially and wheat production needs to be increased by 60% to feed the projected world population of 10 billion by 2050 (Anonymous, 2021b). This increase should come through increasing the cultivated areas on the expense of other crops, which is not realistic, or via improving the cultural practices and genetic gains. Wheat yield increase is hindered by biotic (drought, heat, etc.) and abiotic factors (fungi, bacteria, nematodes, etc.) (Dababat et al. 2018). Plant-parasitic nematodes are considered economically important factors affecting wheat yield and its quality. The main plant parasitic nematodes causing damage to the wheat crop are *Heterodera* spp., *Pratylenchus* spp., *Anguina tritici*, *Ditylenchus dipsaci*, and *Meloidogyne* spp., (Dababat et al. 2016, Dababat and Fourie 2018, Tulek et al. 2015). Although wheat gall nematode has been brought under control in many countries, the nematode still exists wherever wheat is grown and remains common in Eastern Europe and in different parts of Asia and Africa (Agrios 2005). The causal agent of wheat seed gall is *Anguina tritici* which causing ear-cockle disease of wheat turning the seed into seed gall. Wheat seed gall nematode infects aboveground plant parts with symptoms usually appearing in wheat seedlings but are most noticeable prior to heading.

This nematode causes both quantitative and qualitative losses in wheat by reducing yield and its acceptability for human consumption due to flour color change. Losses of 50 and 65% were reported in wheat and rye due to infection with the wheat gall nematodes, respectively (Leukel 1924). Tulek et al. (2015)

estimated yield losses caused by *A. tritici* averaging at 55% in wheat cultivars Pehlivan, Selimiye, Gelibolu, and Kate-A (average yields = 3.74 t ha^{-1}) as compared to control plots (average yields = 8.38 t ha^{-1}). This study was aimed to determine the pathogenicity of *Anguina tritici* toward bread and durum wheat cultivars and losses in the yield component associated with the nematode.

Methods

To determine the effect of *A. tritici* on grain number and the reaction of selected bread and durum wheat cultivars, trials on varietal reactions were conducted at the Trakya Agricultural Research Institute in Edirne, Türkiye, during the 2015-2016 growing seasons.

To determine the impact of *A. tritici* on yield characteristic of wheat, a total of 30 cultivars (25 bread and 5 durum wheat) among the widely cultivated cultivars in different regions of Türkiye were used. The seeds of all varieties used in the experiments were sown by hand in seedbed of 1 m row and distributed in a completely randomized block design with 3 replications. The space between rows was 30 cm and each 1-m long row served as a replicate and was planted with a mixture of 100 healthy and 100-galled seeds. At harvest, each row was manually harvested by sickle and separately threshed by using a threshing apparatus. Grains were separated from glume. For each row, healthy and infected grains were counted and recorded. The seed infection percentage was calculated as per this equation: [(Number of infested seed in plot / Total number of seed in plot) × 100]

Seed galls had been collected from the previous year and were used as inoculum. Before sowing, the galled grains were classified according to their size by using the oblong sifter and seeds between the range of ≥2.0 and <2.2 were used. Then, 10-galled grains were randomly selected and mean number of nematode were determined (11.790 *A. tririci* per grain).

Infected seed ratio and measured grain data were subjected to Analysis of Variance (ANOVA) using Jump 5.0.1 statistical program. The means were compared by a least significant difference (LSD $P < 0.05$) if the F value was significant.

Results

During vegetative and generative stages, deformations on leaves and spikes were extensively observed on plants in the infested plots. At harvest, galled grains occurred extensively throughout infested plots.

The highest infected seed rate among the tested durum and bread wheat cultivars was obtained by Zühre and Kenanbey at a ratio of 24.9% and 71.4%, respectively. Seed gall was formed in all studied cultivars. *A. tritici* made significant damage on wheat cultivars and it was variable among cultivars (Table 1).

The highest infected grain ratio was obtained from the bread wheat Kenanbey (71.4%), whereas the lowest infected grain ratio was revealed in Karahan cultivar (0.2%). Considering the infected grain ratio and healthy grain number, Kenanbey was found to be highly susceptible to *A. tritici*. The wheat cultivar 'Bayraktar-2000' with a seed gall ratio of 59.2% resulted in 1325 healthy grain per plot. In contrast, the seed gall ratio of

0.2% in Karahan-99 resulted in only 750 healthy grains per plot. The seed gall nematode adversely affected Karahan-99 during the vegetative growth period and reduced tillering, stem elongation, and spike formation. In Çeşit-1252, 3531 healthy grains were obtained and the seed gall ratio was only 0.4%

Table 1. Reaction of wheat cultivars to *Anguina tritici* by infected grain rates (%) and average number of healthy grains in one meter planting row

Cultivar	Percentage of infected seed (%)	Cultivar	Number of healthy seed
Kenanbey	71.4a[1]	Basribey-95	3830a[1]
Bayraktar-2000	59.2a	Çeşit1252-	3531a
Demir-2000	30.9b	Ceyhan-99	2587b
Basribey-95	28.1bc	Adana-99	2065bc
Tosunbey	27.9bd	Zühre	1883cd
Zühre*	24.9be	Kızıltan-91	1642ce
Aldane	22.2bf	Gönen-98	1576cf
Sönmez-2001	21.8bf	Kate A-1	1520cf
Kaşifbey-95	21.4bf	Kaşifbey-95	1413cg
Kızıltan-91*	20.8bf	Pandas	1381ch
Ceyhan-99	20.1bf	Bayraktar-2000	1325dh
Kate A-1	19.9bf	Cumhuriyet-75	1274dh
Adana-99	19.6bf	Aldane	1228dh
Selimiye	18.4bf	Pehlivan	1202di
Gerek-79	16.8bf	Cemre	1099ei
Konya-2002	13.4bf	Konya-2002	1054ei
Fırat-93*	12.4bf	Fırat-93	1036ei
Cumhuriyet-75	11.2bf	Bereket	1014ei
Pehlivan	10.4bf	Selimiye	951fi
Altay-2000	9.9bf	İkizce96-	895fi
Bereket	9.8bf	Bezostaja-1	893fi
Cemre	6.9cf	Tosunbey	777gi
Bezostaja-1	6.1cf	Eminbey	775gi
Pandas	5.8cf	Karahan-99	750gi
İkizce96-	5.0df	Altay-2000	745gi
Gönen-98	4.6ef	Gerek-79	712hi
Eminbey*	3.7ef	Gün-91	539ij
Çeşit1252-*	0.4f	Kenanbey	454j
Gün-91	0.4f	Sönmez-2001	445j
Karahan-99	0.2f	Demir-2000	415j
$LSD_{0.05}$	22.9		687

*Durum wheat cultivar

[1] Similar letters within the same column are not significantly different based on LSD test (P>0.05).

Discussion

The wheat gall nematode affects the yield in two ways; firstly, galled grains are a direct yield loss and secondly, the deformation caused by the nematode during the vegetative growth stage inhibits stem elongation and decreases spike number/m^2, the consequences of adversely affecting yield.

Infected grain rate generally reveals the cultivar reaction to *A. tritici*. However, we believe that this should not be the only criterion in determining the susceptibility/resistance or tolerance/intolerance reactions of wheat cultivars to *A. tritici*. Effects on vegetative growth can impact total yield without affecting the percent of galled seeds. In a pot experiment (Ami et al. 2015), seed galls did not occur in wheat cultivars Abae-95 and Rezgare even though nematode symptom were observed. In some wheat cultivars, resistance is manifested as *A. tritici* being unable to complete its life cycle after climbing (Taher 2012). Parveen et al. (2003) found wheat varieties HD-2009 and WH-542 were resistant to *A. tritici* with grains were free from infection although the varieties exhibited symptoms of twisting and crinkling of the leaves which are indicators of the seed gall nematodes feeding. Mohamedova and Piperkova (2013) carried out pot experiments in order to determine resistance to *A. tritici* with five bread wheat; Enola, Aglika, Crystal, Albena and Diamond and their results showed that the highest number of seed galls was formed on the varieties Enola and Aglika followed by Albena and Diamond; 27, 24, 20 and 19 galls per plant, respectively. Ami and Taher (2014) indicated that the highest infection among durum and bread wheat cultivars were obtained from Arey and Maxipak cultivars (37.12 %), (65.33%), respectively.

Wheat seed gall nematode is found in farms where certified seeds are not used and where no seed-cleaning unit is employed. Tülek et al. (2017) found only 13 of 685 wheat seed samples from farmer stocks was infected with seed gall nematode in 2015 in the Trakya Region, Türkiye. The negative impact of *A. tritici* on grain yield and yield traits cannot be neglected, and the farmers in the region must be trained and encouraged to use certified wheat seeds as well as monitoring the nematode in farmers' wheat stocks. Another approach to manage losses in wheat to *A. tritici* is to generate resistant or tolerant cultivars using genetic resources that are available within the wheat germplasms.

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Investigation of resistance of some Moroccan wheat lines against the root-lesion nematodes (*Pratylenchus thornei*)

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Summary

The root lesion nematode *Pratylenchus thornei* causes high yield losses in rainfed wheat fields in Morocco, as well as worldwide. Growing resistant varieties is one of the most effective methods for controlling nematodes. Therefore, a collection of 69 wheat lines (*Triticum aestivum* and *T. durum*), provided by CRRA-Meknes, Morocco and ICARDA, were screened for resistance to *P. thornei* in tubes ($15 \times 20 \times 120$ mm^3) under greenhouse conditions. The resistance level was evaluated based on the numbers of nematodes extracted from roots and soil 9 weeks after infestation. Three lines L3 (DW-37), L14 (DW-37), and L54 (USG3535) were found moderately resistant (Reproduction factor <1) to *P. thornei*

Introduction

Among cereal crops, wheat (*Triticum aestivum* and *T. durum*) is occupying a decent position regarding production, nutrition source, and acreage patterns, especially in developing countries (Nicol et al. 2011). In 2020-21 the wheat global production is estimated to exceed 768.49 million metric tons (USDA 2020). Wheat has prominent adaptability to various geo-climatic conditions as well as dietary traditions as it grows exclusively under both irrigated and non-irrigated areas (Dababat et al. 2015). Around 12.6% of the global annual wheat yield loss is attributed to plant-parasitic nematodes damage, which represents an annual monetary loss of 216 billion US $ (Nyaku et al. 2017). In cereals, plant-parasitic nematodes mostly belong to two groups, viz. root-lesion nematodes (RLN, *Pratylenchus* spp.) and cereal cyst nematodes (CCN, *Heterodera* spp.).

Root lesion nematodes (RLN) are widespread and considered as one of the most important groups of plant-parasitic nematodes in the world (Castillo and Vovlas 2007). In Morocco, *Pratylenchus* spp. constitutes the most important group of PPN in different wheat growing areas (Mokrini et al. 2016). Two species of RLN, viz. *P. thornei* and *P. penetrans* were identified in different wheat producing regions of Morocco (Mokrini et al. 2016).

Many strategies have been developed to manage the RLN occurrence, including chemical control, cultural practices and use of resistant wheat lines (Dababat et al. 2019; Mokrini et al. 2019). The use of resistant and tolerant wheat cultivars is considered one of the most eco-environmental and promising methods for managing RLN in different cropping systems (Nicol et al. 2011; Mokrini et al. 2019).

The objective of this study was to investigate the resistance of wheat lines against the root-lesion nematodes, *Pratylenchus thornei*.

Methods

Germplasm and selection

Sixty-nine wheat germplasm collection provided by INRA-CRRA-Meknes and ICARDA-Rabat were screened for resistance against the root-lesion nematodes (*Pratylenchus thornei*) (Table 1). The set of germplasm represented a collection of 40 lines of durum wheat (CRRA-Meknes) and 29 lines of both durum and winter wheat (ICARDA-Rabat). Seeds were surface sterilized with 3% sodium hypochlorite and rinsed several times in sterilized distilled water, then placed in sterilized Petri dishes with moist blotting paper and left to germinate for 3 days at 23 °C (Figures 1, 2). Seeds were left until 1-2 cm long radicle roots were formed. A single seedling with 3 seminal roots was transplanted in each plastic tube (15 × 20 × 120 mm) containing a potting mixture of sterilized sand, and field soil (70:30 v/v). Sand and field soils were sterilized at 110 °C for 2 h and organic fertilizer was sterilized at 70 °C for 5 h. The experiment was performed to investigate resistance performance against *P. thornei*. Four replicates of each entry were arranged in a completely randomized design in a greenhouse with temperatures between 22°C and 24°C. Plants were sprayed daily with water using an atomizer. Two standard durum wheat lines, viz: CROC_1/AE.SQUARROSA (224)//OPATA, and Ourgh were chosen as check lines (Lr, Ls) for their recognized resistance and susceptibility to *P. thornei*, respectively (Mokrini et al. 2018).

Figures 1 and 2. Seeds were surface sterilized with 3% sodium hypochlorite and rinsed several times in sterilized distilled water, then placed in sterilized Petri dishes with moist blotting paper and left to germinate for 3 days.

Nematode inoculum

Experiments were carried out using one populations of *P. thornei*, collected from Zaers region of Morocco. This population was extracted from wheat roots and soils collected in 2020 from two wheat fields known for historical RLN infection in Zaers region of Morocco. This population was maintained in vitro on carrot-disc cultures according to Moody et al. (1973) after their morphological and morphometrical identification. The nematodes were extracted from carrot disks using modified Baermann method (Hooper 1986). To obtain a uniform population of *P. thornei*, the inoculum of this population was further processed using a 20-µm sieve to separate eggs from juveniles. Nematode suspensions, containing all vermiform stages of *P. thornei*, were prepared using tap water. One week after planting, each seedling was inoculated with a nematode suspension containing 400 individuals of either *P. thornei* (Toktay et al. 2012). The inoculum suspension was transferred into three 2- cm deep holes 0.5 cm distanced from the seedling. The plants were kept in the growth chamber for 9 weeks.

Table 1. The list of the lines evaluated in this study plus the two check lines with their resistant and tolerant reactions. Abbreviations stand for: R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible, S = Susceptible, HS = Highly Susceptible

Code	Line	Type of Wheat (DW/ ?)	Institution	Reproduction factor *(RF)*	Resistance Reaction
L1	21 DW-01	DW	INRA-Meknes	2,29 ± 0.3	MS
L2	21 DW-02	DW	INRA-Meknes	4,69 ± 0.37	HS
L3	21 DW-03	Dur	INRA-Meknes	0,5 ± 0.2	R
L4	21 DW-04	DW	INRA-Meknes	3,01 ± 0.6	S
L5	21 DW-05	DW	INRA-Meknes	2,28 ± 0.2	MS
L6	21 DW-06	DW	INRA-Meknes	3,27 ± 0.4	S
L7	21 DW-07	DW	INRA-Meknes	3,98 ± 0.2	S
L8	21 DW-08	DW	INRA-Meknes	3,11 ± 0.5	S
L9	21 DW-09	DW	INRA-Meknes	2,67 ± 0.4	MS
L10	21 DW-10	DW	INRA-Meknes	5,34 ± 0.4	HS
L11	21 DW-11	DW	INRA-Meknes	2,33 ± 0.3	MS
L12	21 DW-12	DW	INRA-Meknes	7,14 ± 0.4	HS
L13	21 DW-13		INRA-Meknes	3,3 ± 0.4	HS
L14	21 DW-14		INRA-Meknes	0,54 ± 0.1	R
L15	21 DW-15	DW	INRA-Meknes	5,19 ± 0.3	HS
L16	21 DW-16	DW	INRA-Meknes	2,71 ± 0.3	MS
L17	21 DW-17	DW	INRA-Meknes	5,91 ± 0.3	HS
L18	21 DW-18	DW	INRA-Meknes	2,77 ± 0.3	MS
L19	21 DW-19	DW	INRA-Meknes	2,03 ± 0.2	MS
L20	21 DW-20	DW	INRA-Meknes	3,58 ± 0.1	S
L21	21 DW-21	DW	INRA-Meknes	2,05 ± 0.3	MS
L22	21 DW-22	DW	INRA-Meknes	5,21 ± 0.2	HS
L23	21 DW-23	DW	INRA-Meknes	7,29 ± 0.5	HS
L24	21 DW-24	DW	INRA-Meknes	2,37 ± 0.3	MS
L25	21 DW-25	DW	INRA-Meknes	3,52 ± 0.1	S
L26	21 DW-26	DW	INRA-Meknes	3,78 ± 0.4	S

L27	21 DW-27	DW	INRA-Meknes	2,22 ± 0.2	MS
L28	21 DW-28	DW	INRA-Meknes	4,57 ± 0.2	HS
L29	21 DW-29	DW	INRA-Meknes	5,45 ± 0.3	HS
L30	21 DW-30	DW	INRA-Meknes	3,52 ± 0.1	S
L31	21 DW-31	DW	INRA-Meknes	4,58 ± 0.1	HS
L32	21 DW-32	DW	INRA-Meknes	3,11 ± 0.2	S
L33	21 DW-33	DW	INRA-Meknes	4,22 ± 0.1	HS
L34	21 DW-34	DW	INRA-Meknes	1,7 ± 0.2	MR
L35	21 DW-35	DW	INRA-Meknes	4,04 ± 0.2	HS
L36	21 DW-36	DW	INRA-Meknes	2,84 ± 0.3	MS
L37	21 DW-37	DW	INRA-Meknes	2,99 ± 0.5	MS
L38	21 DW-38	DW	INRA-Meknes	4,49 ± 0.4	HS
L39	21 DW-39	DW	INRA-Meknes	2,04 ± 0.3	MS
L40	21 DW-40	DW	INRA-Meknes	3,35 ± 0.3	S
L41	1265		ICARDA-Rabat	5,04 ± 0.3	HS
L42	1256		ICARDA-Rabat	4,56 ± 0.3	HS
L43	Icamor		ICARDA-Rabat	2,31 ± 0.1	MS
L44	Dha Nass		ICARDA-Rabat	2,53 ± 0.5	MS
L45	BT7		ICARDA-Rabat	3,03 ± 0.2	S
L46	BD5		ICARDA-Rabat	1,67 ± 0.2	MR
L47	Florence		ICARDA-Rabat	4,16 ± 0.5	HS
L48	Kharoba		ICARDA-Rabat	3,08 ± 0.1	S
L49	BT33		ICARDA-Rabat	4,38 ± 0.3	HS
L50	1257		ICARDA-Rabat	3,81 ± 0.3	S
L51	1255		ICARDA-Rabat	3,27 ± 0.1	S
L52	BD3		ICARDA-Rabat	6,69 ± 1.3	HS
L53	Parula		ICARDA-Rabat	3,09 ± 0.2	S
L54	USG3535		ICARDA-Rabat	0,79 ± 0.1	R
L55	1179		ICARDA-Rabat	3,98 ± 0.1	S
L56	1258Bidri		ICARDA-Rabat	4,91 ± 0.4	HS
L57	DW5004		ICARDA-Rabat	5,24 ± 0.3	HS
L58	Sumia3		ICARDA-Rabat	4,95 ± 0.3	HS
L59	1267		ICARDA-Rabat	7,45 ± 0.4	HS
L60	1266		ICARDA-Rabat	4,96 ± 0.3	HS
L61	Aguilal		ICARDA-Rabat	3,68 ± 0.2	S
L62	Nax2-BW5907		ICARDA-Rabat	6,34 ± 0.5	HS
L63	BT8		ICARDA-Rabat	4,63 ± 0.3	HS
L64	Nax1-BW5020		ICARDA-Rabat	6,07 ± 0.3	HS
L65	MGB61272		ICARDA-Rabat	3,7 ± 0.1	S
L66	SF 74		ICARDA-Rabat	2,19 ± 0.1	MS

L67	MGB61195		ICARDA-Rabat	2,87 ± 0.3	MS
Lr	CROC_1/ AE.SQUARROSA (224)//OP		CYMMIT	0,3 ± 0.08	R
Ls	MOurgh		Morocco	4,09 ± 0.2	HS

Assessment of resistance

Plants were harvested nine weeks after inoculation and aboveground plant parts were removed. The soil was then removed from the roots by gently shaking the plants. Nematodes were extracted from soil and roots using a modified Baermann method (Hooper 1986). The roots were washed separately for every plant. Nematodes were released from the roots by cutting the root system in 2-cm pieces and macerating them in water for 1 min at high speed in a commercial blender. The reproduction factor (*RF*) of *P. thornei* was determined for each line by dividing the final nematode population (*Pf*) by the initial nematode population (*Pi*).

$$RF = PF/PI$$

The final population of each line was determined from the total number of vermiform stages of *P. thornei* extracted from both the soil and roots of each tube at harvest. The initial population in this study was 400 vermiform stages of *P. thornei* per tube.

The resistant reaction was assessed according to five distinctive groups which were: Resistant (R) = *RF* equal or less than 1; Moderately Resistant (MR) = *RF* between 1-2, slightly more nematodes than in a resistant check; Moderately Susceptible (MS) = *RF* between 2-3, significantly more nematodes than in a resistant check, but not as many as in the susceptible check; Susceptible (S) = *RF* between 3-4, same nematode numbers as of the susceptible check; and Highly Susceptible (HS) = *RF* more than 4, more nematodes than in the susceptible check (Dababat et al. 2016).

Statistical analysis

Data were processed using analysis of variance (ANOVA) after being normalized by the Anderson–Darling normality test (Stephens, 1974). Protective Least Significant Difference (LSD) test was adopted to detect significant differences between lines at $P < 0.001$ via SPSS software V 17.0 (SPSS Inc., Chicago, IL, USA). Population structure was distinguished by the Linear Discriminant Analysis (LDA) to determine putative groups of wheat lines based on their resistance, susceptibility to *P. thornei*.

Results

The 67 wheat lines collection provided by INRA-CRRA-Meknes and ICARDA-Rabat were assessed for their host status against *P. thornei* based on the final population of nematodes extracted from both soil and root (Table 1). Nine weeks after inoculation, the reproduction factor of *P. thornei* in the 67 lines of wheat ranged from 0.5 to 7.4 (Figs. 1 and 2; Table 1). Reaction attributes ranged from resistant (L3, *RF* = 0.5; L14, *RF* = 0.54; L54, *Rf* = 0.79) to highly susceptible (*RF* > 4) (Figs. 1 and 2; Table 1). The *RF* values of both check lines ranged from 0.33 (Lr) to 4.1 (Ls). Lines L3, L14, and L54 were the most resistant, with *RF* values of 0.5, 0.54 and 0.79, respectively (Fig. 2);

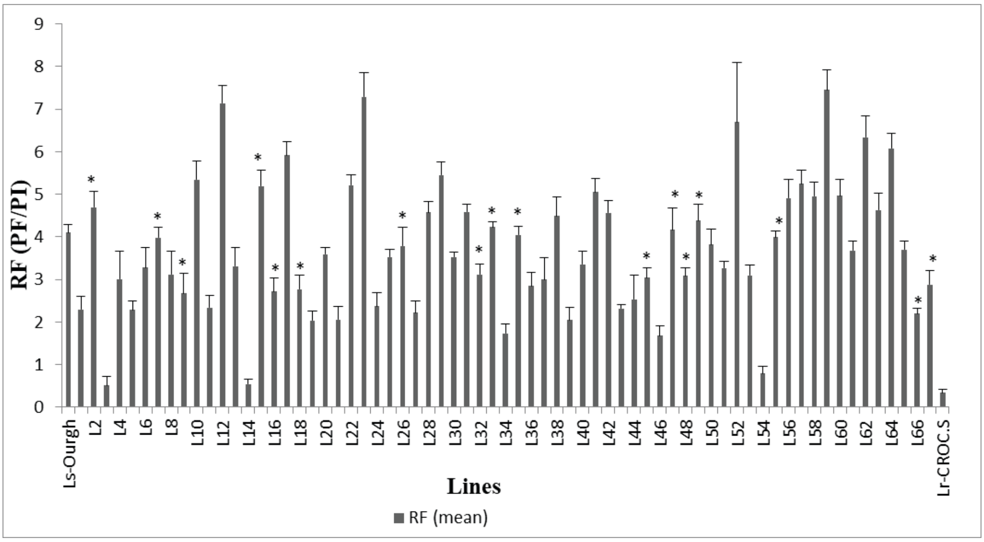

Figure 3. *Pratylenchus thornei* reproduction factor (*RF*), maintained in growth chamber experiments. Stars represent homogenous groups based on the LSD test for each line at $P < 0.001$. Error lines represent the standard error (SE) (n = 4).

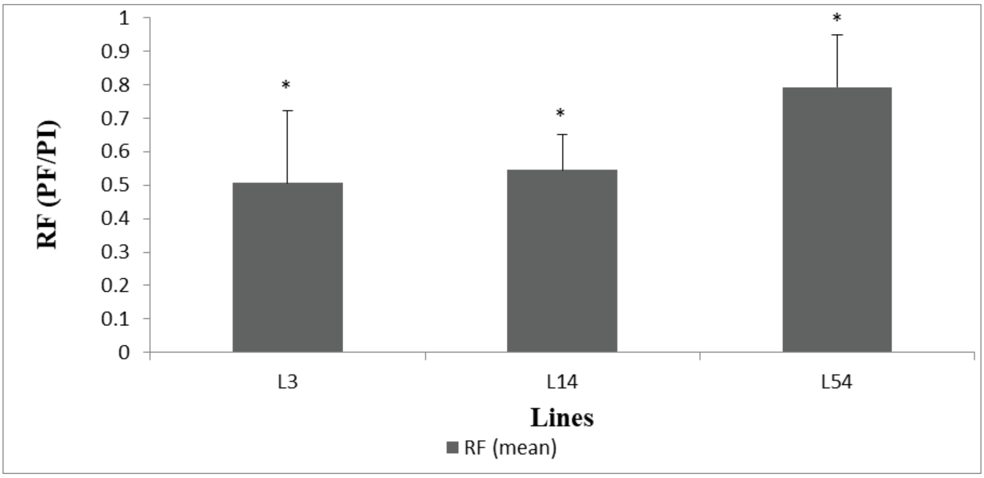

Figure 4. *Pratylenchus thornei* reproduction factor (*RF*) per resistant lines of wheat, 9 weeks after nematode inoculation with 400 vermiform stages of *P. thornei.*

Population structure based on Linear Discriminant Analysis (LDA) displayed five distinct groups, depicting the resistance reaction against *P. thornei* among the 67 lines evaluated (excluding both check lines) (Figure

3). The first two groups consist of 3 resistant (R) lines, including L3, L14, and L54, followed by 4 moderately resistant (MR) lines, including L34 and L46. The third group comprises 15 moderately susceptible (MS) lines, including (L1, L5, L9, L11, L16, L18, L19, L21, L24, L27, L36, L39, L43, L44, L66 and L67. Eighteen susceptible lines (S) were determined, followed by 27 highly susceptible lines.

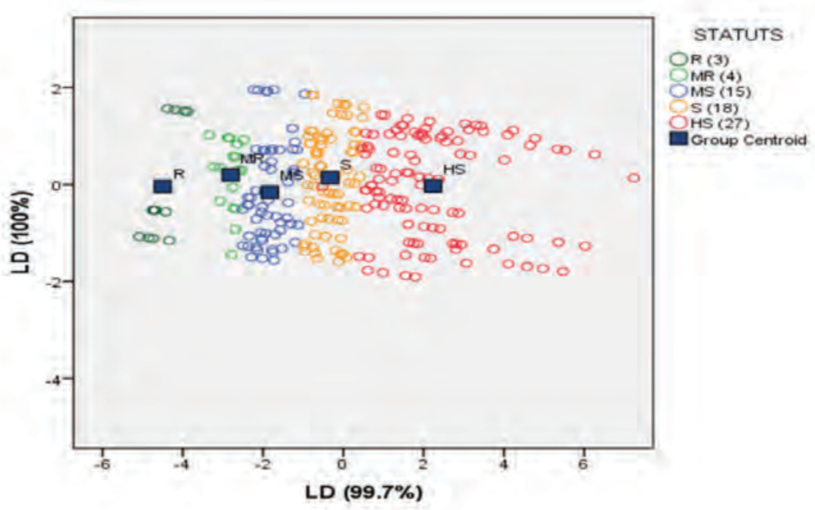

Figure 5. Linear Discriminant Analysis (LDA) showing the population structure for a set of 67 lines of wheat based on RF values; LDA displaying resistance reaction (RR) ranking to *Pratylenchus thornei.*

Conclusion

In conclusion, three lines of wheat *viz.*, L3 (DW-37), L14 (DW-37), and L54 (USG3535) were resistant to the root-lesion nematodes, *P. thornei.* This study provides baseline insights for selecting genuine Moroccan wheat lines for future breeding and disease management programs involving resistant and tolerant attributes. However, the field performance of these lines against *Pratylenchus thornei* attacks should be evaluated before they are released to the farmers to confirm their resistant.

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Plant-parasitic nematodes associated with cereals in Sakarya, Turkey

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Summary

Sakarya is one of the important provinces in Turkey where wheat, maize and potato production are carried out intensively. Plant parasitic nematodes (PPN) particularly the root lesion nematodes, *Pratylenchus* spp. and the cereal cyst nematodes, *Heterodera* spp. are known to be very damaging on the cereal the cropping system. The recent study appraised the occurrence and prevalence of PPNs and identified the cereal cyst nematode, *Heterodera* spp. population in the most important cereal growing areas in the Hendek, Pamukova, Geyve, Akyazı and Central districts in Sakarya Province. The result showed that PPNs were detected in 92% of soil samples of which 13 genera were identified from the soil samples collected from the surveyed areas including important genera of PPN; *Pratylenchus, Heterodera, Geocenamus, Ditylenchus, Helicotylenchus* or *Rotylenchus, Paratylenchus, Pratylenchoides* and *Tylenchorhynchus*. The *Heterodera* species, *H. filipjevi* was identified by using morphological/morphometrical and molecular analysis and it was found in 44% of the soil samples. Within the morphological and molecular assays, intraspecific polymorphism was not observed within *H. filipjevi* populations which, then, could be in the same group. This is the first comprehensive report on *H. filipjevi* from wheat cropping areas in the Sakarya province. The results showed that there was a high infestation rate of *Heterodera filipjevi* recorded from Geyve and Pamukova followed Hendek and Akyazı, however, a low infestation rate was detected Central district. Intensified cereal cropping systems with/without non-cereal rotations increased the risk of plant parasitic nematodes especially the root lesion nematode, *Pratylenchus* spp. and Cereal cyst nematode; *Heterodera filipjevi* infection of wheat production areas of Sakarya Province.

Keywords: Wheat, nematodes, identification, occurrence, frequency

Introduction

Soil-borne diseases, caused by fungi and nematodes, are major yield-limiting factors and they are difficult to control. Plant parasitic nematodes (PPN) alone, have been calculated to take away approximately 10% of the World's global agricultural output, causing economic losses valued at over $125 billion each year (Chitwood 2003). Plant parasitic nematodes are considered as economically important pests of a wide range of plants including ornamentals, vegetables, and fruit trees (Urwin et al. 2002). The most devastating and economically damaging group of PPNs are sedentary endoparasites (Sasser and Freckman 1987; Koenning et al. 1999; Chitwood 2003; Jones et al. 2013). Cyst nematodes, as a group of endoparasitic nematodes, cause high yield losses and therefore have been studied intensively. Many studies focus on the interaction between cyst nematodes and their host plant and try to elucidate their biology. Cyst nematodes including the genera *Heterodera* spp. and *Globodera* spp. infect a wide range of host plants like vegetables, legumes and cereals. The cereal cyst nematode, *Heterodera* spp. is the only a pest on the cereal crops and consisting of 12 valid species and with overlapping morphological characters that make them difficult to distinguish from one another (Umarao et al. 2013; Dababat and Fourie 2018). The identification of *Heterodera* species using morphological and morphometrical characteristics is time consuming and requires great skill and training by the observers. However, there are many other characteristics allowing to discriminate between different species of nematodes e.g. molecular tools. The non-coding internal transcribed spacer sequences, ITS1 and ITS2 including 5.8S region of ribosomal DNA (rDNA) has been very useful for the accurate identification of the species and characterization of molecular genetic variation within the species of plant parasitic nematodes (Subbotin et al. 2003, 2018).

In different parts of Turkey, *Heterodera* spp. populations obtained from different wheat cropping systems have mainly been identified based on their morphology/morphometrics and molecular tools (Sahin et al. 2009; İmren et al. 2012, 2015, 2018; Toktay et al. 2015). However, very little information is available on the diversity and variability of their morphometrics and genetics in Marmara Region especially in Sakarya Province. Thus, this research has been conducted to study the status of PPNs including the *Heterodera* spp. in the wheat growing areas in Sakarya Province where an intensive survey was conducted in the major wheat growing areas of Hendek, Pamukova, Geyve, Akyazı and Central districts in Sakarya Provinces, Turkey. The main objectives of this study were to: (a) prove the occurrence and prevalence of the major genera of PPNs (b) identify and compare both cysts and second-stage juveniles (J2s) of populations of *Heterodera avenae* group using morphological, morphometrical and molecular approaches including species-specific polymerase chain reaction (PCR) and sequencing of the ITS-rDNA expansion segments, and (c) determine the phylogenetic relationships between these populations.

Methods

The survey was conducted in 2018, at the maturity stage / harvesting time of the cereals, from Hendek, Pamukova, Geyve, Akyazı and Central district in Sakarya Province (Figure 1). Surveys had been undertaken at the heading and flowering time of wheat for migratory nematodes and the harvesting time of wheat for sedentary nematodes. Totally 56 soil samples were taken randomly in a zigzag pattern, sampling sites were within a 10-20 km distance from each other. A minimum of 7-10 cores were taken per sample, then soil mixed and a representative of 2 kg soil was taken as a sub-sample for further analysis.

Migratory nematodes were extracted from 100 cm^3 of soil by using a modified "Petri-dish" Baermann funnel method (Hooper 1986). Extracted nematode was transferred to graduated cylinders which allowed to settle for 8 h, supernatant discarded and the nematodes/debris were poured into 15 ml tubes. Migratory nematodes were counted under a light microscope at 100 × magnification and identified to the genus level.

Sedentary nematodes (*Heterodera* spp.) were extracted from 200 cm^3 of soil by using the modified sieving–decanting method using a 60 mesh (0.25 mm) and a 100-mesh (0.015 mm) sieves (Fenwick 1940; Dababat et al. 2014). At least 10 full cysts were selected and hand-picked from each sample and stored at 4 °C for later use in the molecular and morphological analysis. Sedentary nematodes were morphologically identified to genus level under a stereo-binocular microscope (Zeiss, Jena, Germany, and V20). The incidence of the nematode (number of samples with nematode / total number of samples) was calculated for each field.

Figure 1. Plant parasitic nematode survey locations in Sakarya Province.

The nematodes were killed in a hot water bath and fixed in 2 percent formaldehyde. Nematodes were processed for dehydration by Seinhorst's method and mounted on glass slides in anhydrous glycerin (Seinhorst 1959). Identification of nematode genera and species were based on the morphology with the help of keys (Handoo and Golden 1989; Siddiqi 2000; Handoo 2002). All nematodes except the cyst nematode, *Heterodera* species were identified to genus level based on key morphological features (Siddiqi 2000, Handoo 2000, Handoo et al. 2007). The exact number of nematodes in each genus was assessed. The cyst nematodes by using morphological traits and molecular markers (ITS, 28 S and 18 S rDNA gene sequences).

Results and discussion

The whole survey area represented 22.6% of the barley and wheat hectarage in Sakarya Province. Plant-parasitic nematodes were found in 90% of soil samples. Thirteen genera of plant-parasitic nematodes were identified, including *Heterodera*, *Merlinus*, *Pratylenchus*, *Pratylenchoides*, *Amplimerlinus*, *Helicotylenchus*, *Paratylenchus* and *Tylenchorhynchus* (Table 1). Additionally, free-living nematodes were detected in all soil and root samples.

Among the plant-parasitic nematodes, *Heterodera* and *Pratylenchus* species had a very low occurrence, being found in 22% and 26% of the samples, respectively. Additionally, cyst and root lesion nematodes were found together in the same location in 38% of the sampling areas. Frequency of the other important genera, *Merlinus*, *Pratylenchoides*, *Amplimerlinus*, and *Helicotylenchus* were 28, 30, 32, and 40%, respectively (Table 1).

The results of the present survey confirm that *Pratylenchus* are among the most important parasitic nematode associated with wheat fields in Sakarya Province. It was also widely distributed, with a mean density of 240 juveniles and adults (range of 400–980) per 100 cm^3 of soil (Table 1). *Pratylenchus* populations were found in 45% of the soil samples collected from wheat fields in 18 different locations of Sakarya Province. This lesion nematode has a wide host range including cereals, legumes, fruits, and vegetables (Hunt et al. 2005). The extensive nature of agriculture in the study area would therefore favour to build higher populations of this nematode. *Pratylenchus* spp. is a common pest in agronomic settings and is responsible for significant yield losses worldwide (Castillo and Volvas 2007). In Turkey for example, it is the most important nematode parasite of Turkey, and it has been identified in wheat growing locations in Turkey (Dababat et al. 2014; Toktay et al. 2015).

Table 1. Occurrence of plant parasitic nematode stages associated with wheat in Sakarya province

Genus of Nematode	Proportion of infested fields (%)	Number of nematodes
Heterodera	22	
Merlinus	28	960 ± 120 (220 - 1100)
Trophurus	16	240 ± 20 (200 - 360)
Paratrophurus	14	140 ± 40 (80 - 980)
Pratylenchus	26	240 ± 40 (400 - 980)
Amplimerlinus	32	340 ± 60 (200 -880)
Helicotylenchus	40	420 ± 50 (280 - 780)
Tylenchus	34	320 ± 40 (200 - 480)
Pratylenchoides	30	640 ± 160 (200 - 980)
Scutylenchus	28	550 ± 60 (100 - 860)
Filenchus	24	280 ± 20 (100 - 200)
Boleodorus	20	120 ± 160 (140 - 360)
Basiria	16	240 ± 30 (160 - 900)

For *Heterodera*, we found an average of 8 cysts (range of 2-28) in 250 cm^3 of soil. Also, the average eggs/cyst conservatively would be 180, and the range of number of eggs or juveniles of *Heterodera* would be 22-82 per g of soil. However, *Heterodera*, was reported in low number and frequency in both root and soil samples in Sakarya province. It is a potential pest of wheat having been reported in Turkey (Imren et al. 2012; 2015). Three species *Heterodera* have been associated with Gramineae family in various parts of the world (Imren et al. 2012, 2015; Toktay et al. 2015; Dababat and Hendrika 2018, Toktay et al. 2020). This is the first report of a *Helerodera* species on wheat in Sakarya. This nematode may be *H. filipjevi*, the cereal cyst nematode which occurs in temperate, and subtropical and is an important pathogen of wheat worldwide (Swarup and Sosa-Moss 1990). In Turkey, *H. filipjevi* has only been reported from some semi-arid regions of South Anatolia (Toktay et al. 2015) and Central Anatolia (Sahin et al. 2008).

Several of the parasitic nematode species found during the present survey are potential pathogens of wheat. The influence of at least the predominant species, especially *Pratylenchus* and the *Heterodera* species, on the growth and yield of this important crop in Turkey will have to be established. The population densities of *Pratylenchus* averaged 240 individuals/100 g soil in the Pamukova in Sakarya Province. Although numerous abiotic and biotic factors will determine the extent of the damage caused by *Pratylenchus* the observed population densities are high enough to suspect damage.

This study highlighted the eco-regional distribution of the main genera of plant parasitic nematodes with an emphasis on the cereal cyst nematode species *Heterodera* species in Sakarya Province. Further detailed surveys in the Sakarya Province and comprehensive pathotype studies on the *Heterodera* species using populations from different regions are still needed. In conclusion, the recommendations of this survey to the policy makers and the researchers are to; diversify cultivated wheat sorts to include durum wheat where the high cysts existing areas with the durum wheat that is more resistant than spring wheat to cyst nematodes; follow cultural practices especially crop rotation, breed for germplasm with high level of resistant to the cereal cyst nematodes and lastly, to train young capacity on building to work on soil borne disease topics in the region.

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Evaluation of durum wheat from Kazakhstan and Siberia for nematode (*Heterodera filipjevi*) resistance

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Summary

Kazakhstan –Siberian (KASIB) network was established in 2000, it includes 18 spring wheat research and breeding programs.. Spring durum wheat (180 varieties/lines) from KASIB network, including several varieties of triticale from CIMMYT were evaluated for resistance and tolerance to cereal cyst nematode, *Heterodera filipjevi* in the growth chamber. The experiments were carried out in two repetitions in three replications. Replicated small tubes were infested with second-stage juveniles. The genotypes were divided according to resistance and tolerance into 5 groups according to the number of cysts per plant (R, MR, MS, S and HS). A comparison of two repetitions of the experience, as well as a comparison of Kazakhstan and Russian varieties was carried out. Triticale germplasms showed resistance to nematode infectation. Three varieties/lines of durum wheat also showed resistance. Fifteen moderately resistant varieties/lines were identified during this study. Most varieties/lines were identified as susceptible and highly susceptible. However, DArTseq and SNP Analysis are needed for further studies on resistant germplasms.

Introduction

Wheat is a major commodity for export in Kazakhstan and is grown annually more than 12 million hectares (mha) area. The average yield is one ton per hectare. In 2019, a total of 465 thousand hectares of durum wheat were planted in Kazakhstan with an annual production of up to 500 thousand tons (Anuarbek et al. 2020). The durum wheat cultivation area in Kazakhstan in 2020 was estimated at 750,000 ha with the leading regions: North Kazakhstan (300,000 ha), Kostanay (260,000 ha) and Akmola (150,000 ha) (Mal'chikov et al. 2020). Annually wheat yield production in Kazakhstan is very low and the main reason behind this low production is biotic factors. Biotic factors such as nematodea are the main constrain in wheat cultivation areas. The cereal cyst nematode (CCNs; *Heterodera* spp.) is causing a significant cereal crop loss which has been estimated to be up to $80 billion.

According to Toumi et al. (2018) three-major species of Cereal cyst nematodes (CCN) *Heterodera avenae*, *H. latipons*, and *H. filipjevi* are distributed worldwide and cause considerable damage. To date the published studies from various countries have reported that the maximum yield loss due to Plant Parasitic Nematodes (PPNs) causes up to 40-90% of crop loss (Bhatta et al. 2019).

Previously the studies on the presence of nematodes in the soils of Kazakhstan were intensively carried out during the Union of Soviet Socialist Republics, from about 1950 to 1990. Those studies described various types of potato, beet and other types of nematodes were present in Kazakhstan cultivated areas. They were also studied more moderate level of plant breeding for resistance to nematodes. (Uvalieva et al. 2019) After collapsing of the Soviet Union, many studies were suspended and no research was conducted in this area between 1990 and 2015. Studies in the field of cyst nematodes were carried out by Kirjanova and Sagitov (1975) and Kirjanova et al. (1976), for their management purpose. Dutbayev et al. (2015) conducted a study to maintain and increase the yield of wheat, and the results revealed that it is necessary to find resistant varieties to control soil-borne pathogens. They also studied hexaploid syntetic wheat for resistance to CCN. Results showed that twelve lines from Kazakhstan included one that was highly resistant, three that were resistant, and seven that were susceptible.

In 2019 - 2020, survey was conducted to search for PPNs in the main cereal-growing areas in northern Kazakhstan (Dababat et al. 2020). It was found that 44.75% of cereal fields are infested with *H. filipjevi*. The development of resistant varieties against the nematodes is a necessary task for obtaining high sustainable wheat yields (Imren et al. 2021). An excellent source for starting the study of germplasm is the Kazakhstan-Siberian (KASIB) network nursery which are established in 2000, The KASIB-DW nursery presents breeding lines and varieties collected from 2002-2020, provided by various institutes from Russia and Kazakhstan, and includes more than 150 varieties and lines of spring durum wheat (Tajibayev et al. 2021). The purpose of this present study was to evaluate the various durum wheat varieties /lines for resistance and tolerance to *Heterodera filipjevi* (CCN) in the growth chamber.

Methods

Wheat Cultivars: A total of 176 lines KASIB Spring Durum Wheat nursery (KASIB-DW) with 3 varieties of triticale and 2 from CIMMYT genebank (Mexitol, Triticale T6487 -OMI –OMI) and Kazakhstan - Ukrainian variety (UKAZ) were screened for resistance against the *H. filipjevi* (Table 1). Four standard check lines Katea (MR), Sonmez (MR), Kutluk (S), and Bezostaya (S) were used in this study.

Nematode Inoculum: Experiments were conducted in a growth chamber in a randomized complete block design (RCBD) with three replicates in two repetitions. All procedures for nematode inoculation were carried out in the CIMMYT SBDp laboratory in Eskisehir, Türkiye in accordance with their protocol. According to Cobb's decanting and sieving method *H. filipjevi* cysts were extracted (Cobb 1918). The hatched second-stage juveniles were used as inoculation in screening tests. Single wheat seed was planted in standard small tubes (16 cm in height × 2.5 cm in diameter) filled with a sterilized mixture of sand, field soil, and organic matter (70:29:1). The plants were grown in a growth chamber with a 16-h artificial photoperiod and maintained at a temperature of 22 ± 3 °C with 70% relative humidity. Plants were harvested after two months and cysts after extraction from the roots were collected and counted under a stereomicroscope. The genotypes were divided into 5 groups based on the number of cysts per plant, taking into account the reaction of check varieties with known resistance to CCN. The groups were: resistant (R) - as few as or fewer cysts than in the known resistant check; moderately resistant (MR) - slightly more cysts than in the resistant check; moderately susceptible (MS) - significantly more cysts than in the resistant check, but not as many as in the susceptible check; susceptible (S) - as many cysts as in the susceptible check and the number of cysts per root system considered damaging; and highly susceptible (HS) - more cysts than in the susceptible check.

Data were analyzed according to standard analysis of variance procedures using freely available statistical program for Windows R and RStudio with packages "agricolae", "plotrix". Differences among treatments were tested using one way analysis of variance (ANOVA), followed by the Duncan Test for mean comparison if the F-value was significant ($P < 0.05$). Significant differences between the genotype were performed based on the Least Significant Difference (LSD).

Table 1. Characteristics and reaction of the Kazakhstan –Siberain (KASIB) network wheat germplasms screened for resistance to *Heterodera filipjevi*

№	Name	Origin	Institution	1st run cysts	1st run resistance	2nd run, cysts	2nd run resistance	Mean, cysts	Resistance Mean
1	Kostanayskaya 30	Kazakhstan	Karabalyk AERS	8.7	MR	12.7	MS	11	MS
2	160.93	Kazakhstan	Karabalyk AERS	11.7	MS	10.3	MR	11	MS
3	362.91	Kazakhstan	Karabalyk AERS	16	S	13	MS	15	MS
4	6801-34-6	Kazakhstan	KazRIAPG	10	MR	1	R	6	MR
5	654-1-2-3-4	Kazakhstan	KazRIAPG	8.3	MR	6	MR	7	MR
6	17590	Kazakhstan	KazRIAPG	12.3	MS	13.7	MS	13	MS
7	Angel	Russia	Siberian ARI	13.7	MS	14.7	MS	14	MS
8	Ametist	Russia	Siberian ARI	13	MS	15.3	MS	14	MS
9	Omskiy korund	Russia	Siberian ARI	15.3	MS	15	MS	15	MS
10	Gordelforme 430-88	Kazakhstan	Karabalyk AERS	9.3	MR	14	MS	12	MS
11	Gordeiforme 362-91	Kazakhstan	Karabalyk AERS	14.3	MS	11	MS	13	MS
12	Gordeiforme 242-93	Kazakhstan	Karabalyk AERS	15.3	MS	13.7	MS	15	MS
13	Gordeiforme 94-71	Russia	Siberian ARI	13.3	MS	12.7	MS	13	MS
14	Gordeiforme 91-102-6	Russia	Siberian ARI	13.7	MS	13.7	MS	14	MS
15	Gordeiforme 91-22-2	Russia	Siberian ARI	19.7	HS	5.7	MR	13	MS
16	Kargala 18	Kazakhstan	Aktobe AERS	12.3	MS	13.3	MS	13	MS
17	452-MC	Kazakhstan	Aktobe AERS	12.3	MS	15.7	S	14	MS
18	Gordeiforme 91-144-4	Russia	Siberian ARI	15.7	S	14.3	MS	15	MS
19	Altayskaya Niva	Russia	Altay ARI	3.3	R	4	R	4	R
20	Gordeiforme 415	Russia	Altay ARI	14.3	MS	12.3	MS	13	MS
21	Gordeiforme 417	Russia	Altay ARI	13	MS	13.3	MS	13	MS
22	Asangali	Kazakhstan	Karabalyk AERS	13.7	MS	17	S	15	MS
23	Kargala 303	Kazakhstan	Aktobe AERS	13.7	MS	13.3	MS	14	MS
24	Nauryz 8	Kazakhstan	KazRIAPG	15	MS	14.7	MS	15	MS
25	Toma	Kazakhstan	KazRIAPG	11.7	MS	13.3	MS	13	MS
26	Seymur	Kazakhstan	KazRIAPG	13.7	MS	14	MS	14	MS
27	Karabalykskaya chernokolosaya	Kazakhstan	Karabalyk AERS	15.3	MS	12	MS	14	MS
28	Altyn Dala	Kazakhstan	Karabalyk AERS	15.3	MS	12.3	MS	14	MS
29	Gordeiforme 426	Russia	Altay ARI	10.3	MR	14.7	MS	13	MS
30	Gordeiforme 94-94-13	Russia	Siberian ARI	16	S	12.7	MS	14	MS

31	Kargala 1514/06	Kazakhstan	Aktobe AERS	12.7	MS	15.7	S	14	MS
32	Kargala 1515/06	Kazakhstan	Aktobe AERS	13	MS	14.7	MS	14	MS
33	Kargala 1516/06	Kazakhstan	Aktobe AERS	15.3	MS	14.7	MS	15	MS
34	Altyn Shygys	Kazakhstan	Karabalyk AERS	11.7	MS	12	MS	12	MS
35	Altyn Dala	Kazakhstan	Karabalyk AERS	13.3	MS	14	MS	14	MS
36	Nauryz 6	Kazakhstan	KazRIAPG	7.3	MR	12.3	MS	10	MR
37	Lan	Kazakhstan	KazRIAPG	14.3	MS	15	MS	15	MS
38	Gordeiforme 95-139-4	Russia	Siberian ARI	9	MR	12	MS	11	MS
39	Bolashak	Kazakhstan	Karabalyk AERS	7.7	MR	15.3	MS	12	MS
40	Gordeiforme 97-49-1	Russia	Siberian ARI	10.7	MS	7	MR	9	MR
41	Gordeiforme 98-42-1	Russia	Siberian ARI	13	MS	12.7	MS	13	MS
42	Kargala 1671	Kazakhstan	Aktobe AERS	14	MS	12.7	MS	13	MS
43	Gordeiforme 113/01	Kazakhstan	Karabalyk AERS	8.3	MR	15	MS	12	MS
44	Corona	Kazakhstan	Kaz. Grain Inst.	14	MS	12	MS	13	MS
45	Durum 49	Kazakhstan	Kaz. Grain Inst.	15.3	MS	14.3	MS	15	MS
46	653d - 44	Russia	Samara ARI	13	MS	14	MS	14	MS
47	Omksiy Izumrud	Russia	Siberian ARI	14	MS	15.3	MS	15	MS
48	Omskiy Cirkon	Russia	Siberian ARI	15	MS	15.3	MS	15	MS
49	Line 18404	Kazakhstan	KazRIAPG	12	MS	12	MS	12	MS
50	Line e145-z	Kazakhstan	Kaz. Grain Inst.	12.7	MS	15.7	S	14	MS
51	Line e147-z	Kazakhstan	Kaz. Grain Inst.	7.3	MR	13.7	MS	11	MS
52	Line 18472-3-2	Kazakhstan	KazRIAPG	6	MR	5.3	R	6	MR
53	Sharifa	Kazakhstan	Karabalyk AERS	13	MS	14.3	MS	14	MS
54	Shortandinskaya 256	Kazakhstan	Kaz. Grain Inst.	14.3	MS	15.7	S	15	MS
55	Elizavetinskaya	Russia	South-East ARI	8.7	MR	14	MS	11	MS
56	Valentina	Russia	South-East ARI	9.3	MR	14.7	MS	12	MS
57	Leucurum 1469d-21	Russia	Samara ARI	3.3	R	7.3	MR	5	R
58	Zhemchuzhina Sibiri	Russia	Siberian ARI	11.7	MS	11	MS	11	MS
59	Line 19029	Kazakhstan	KazRIAPG	14.7	MS	15.7	S	15	MS
60	Gordeiforme 69-08-2	Kazakhstan	Kaz. Grain Inst.	13	MS	16	S	15	MS
61	Gordeiforme 2264	Kazakhstan	Karabalyk AERS	16	S	13	MS	15	MS
62	Gordeiforme 2383	Kazakhstan	Karabalyk AERS	14	MS	16	S	15	MS
63	Gordeiforme 881	Russia	Altay ARI	11.3	MS	16.7	S	14	MS
64	Gordeiforme 04-76-5	Russia	Siberian ARI	10.3	MR	18	S	14	MS
65	Gordeiforme 05-42-12	Russia	Siberian ARI	10.7	MS	12.3	MS	12	MS
66	Annushka	Russia	South-East ARI	14	MS	16.7	S	15	MS
67	Leucurum 1469-21	Russia	Samara ARI	14	MS	13.7	MS	14	MS
68	Seymur 17	Kazakhstan	KazRIAPG	10.7	MS	14.7	MS	13	MS
69	Line 69-08-2	Kazakhstan	Kaz. Grain Inst.	12	MS	13	MS	13	MS
70	Kostanayskaya 15	Kazakhstan	Karabalyk AERS	13.7	MS	11.3	MS	13	MS
71	Gordeiforme 1790	Kazakhstan	Karabalyk AERS	11.3	MS	17.7	S	15	MS
72	Line №9	Kazakhstan	Karabalyk AERS	12	MS	9.7	MR	11	MS

73	Line 1693d-71	Russia	Samara ARI	15	MS	13.7	MS	14	MS
74	Celinnaya	Russia	Orenburg ARI	15	MS	15	MS	15	MS
75	Melyana	Russia	Orenburg ARI	13	MS	14.7	MS	14	MS
76	18093-7-2	Kazakhstan	KazRIAPG	11	MS	17.3	S	14	MS
77	Mexitol	Mexico	CIMMYT	3.3	R	4	R	4	R
78	Triticale T6487 -OMI -OMI	Mexico	CIMMYT	4	R	1	R	3	R
79	Triticum Polonicum	Kazakhstan	KazRIAPG	12.7	MS	13	MS	13	MS
80	triticale UKAZ	Kazakhstan	KazRIAPG	5	R	7.4	MR	6	MR
81	Bezostaja	Türkiye	CCN	32.7	HS	34.3	HS	34	HS
82	Katea	Türkiye	CCN	19.3	S	11	MS	15	MS
83	Kutluk	Türkiye	CCN	18	S	33.3	HS	26	HS
84	Sonmez	Türkiye	CCN	15	MS	10.3	MR	13	MS
LSD				1.65		1.24			

Results

The analyses of phenotypic data indicated the significant differences among the genotypes for resistance to *H. filipjevi* and showed a range of responses varying from resistant (R) to highly susceptible (HS) response (Figure 1). Three triticale varieties (two from CIMMYT and one Kazakh-Ukrainian) were resistant to the CCN. Observed durum wheat germplasms from both Kazakhstan and Russia countries are equally resistant (3%), 3 R genotypes (3%), and 15 MR genotypes (8%). Sixty seven varieties/lines were MS (40%), fifty six varieties/lines were S (30%), and forty two varieties/lines were HS (24%). Out of 101 genotypes of durum wheat and triticale of Kazakh varieties/lines one (triticale) R (1%), five genotypes MR (5%), 43 varieties/lines MS (43%). 73 durum wheat genotypes from Russia showed that 3 varieties/lines R (3%), On average, over two repetitions, there were no moderately resistant lines, but in each of the repetitions, some of the lines/varieties showed moderate resistance to CCN. A comparative analysis of the averages showed that most of the varieties and lines from both countries are moderately susceptible, susceptible, and highly susceptible to *H. filipjevi*.

a b c

Figure 1. Resistance of durum wheat varieties/lines to *Heterodera filipjevi*, a) first repetition, b) second repetition c) average of two repetitions.

Discussion

Based on the results of repeated growth chamber tests, the durum wheat varieties/lines grown in the Eskisehir region of Türkiye were identified as a valuable source of resistance to species of CCN, *H. filipjevi*, which are distributed in different regions of Kazakhstan in the fields of wheat production. Studies were carried out on pathotypes found in Türkiye since these pathotypes are more common in Kazakhstan and are not yet known at the moment. Triticale lines from CIMMYT can be used for breeding purposes to improve both wheat and triticale. Also, the triticale variety produced in Kazakhstan together with Ukraine can serve as a forage base without crop losses from CCN.

Assessment of the KASIB network collection for resistance to nematodes showed that varieties and lines collected from Russia and Kazakhstan have resistant and moderately resistant lines to *H. filipjevi*, which can be used as parental donors for resistance to nematodes (Tajibayev et al. 2021). Unfortunately, there are not many lines resistant to nematodes. From this it follows that it is necessary to replenish the collection with nematode-resistant lines. To reinforce phenological observations and more accurately determine resistant varieties and lines of this collection, genetic studies are needed to search for Cre gens using the DarTseq, SNP Analysis and GWAS.

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Preliminary studies on biological control of wheat root rot pathogens

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Summary

This study was carried out to determine the *in vitro* antagonistic potential of some endophyte and rhizosphere bacterial microbiomes against wheat root rot pathogens such as *Fusarium culmorum*, *Drechslera sorokiniana*, *Gaeumannomyces graminis* var. *tritici* (*GGT*), which are highly destructive on wheat production areas and causing important economic losses. Inhibitions of mycelial growth of major foliar and soil borne fungal disease agents were investigated. Totally 141 bacterial biocontrol agents were studied on their biocontrol activities. Among them, 31 bacterial isolates (21.98% of total isolates) inhibited the mycelial growth of *F. culmorum* by 96.1%, *GGT* by 100% and *D. sorokiniana* by 89.9% respectively. Majority of the bacterial isolates were identified as *Bacillus* spp. Certain isolates of *Bacillus* spp. have caused noticeable morphological changes on mycelia of *F. culmorum*, *GGT* and *D. sorokiniana*. Antagonistic potentials of bacterial isolates were found to increase by pre-incubation time prior the fungal inoculation. Due to high antagonistic properties, efficient isolates of *Bacillus* spp. may be used as biocontrol agents against soil borne diseases as an alternative to pesticides to promote sustainable agriculture.

Introduction

Wheat (*Triticum* spp.) is the most produced product in the grain fields of Türkiye. According to 2019 data, wheat is grown on 7.7 million hectares, barley on 2.7 million hectares and corn on 0.7 million hectares of total grain areas in Türkiye. Wheat, which ranks first with a production of 20.6 million tons in cereals with a total production of 35 million tons, constitutes 55.2% of the grain production (Anonymous 2020).

Abiotic and biotic stress factors are the most important factors that negatively affect the yield and quality of wheat. Pathogens, one of the biotic stress factors, cause diseases in plants, affect yield and quality significantly and cause economic losses. Among them, soil-borne pathogens constitute the most important group.

Root and crown disease agents are generally soil-borne. They are also carried by seeds and plant residues. It is very difficult to combat these factors due to their ability to survive in the soil for many years. It is possible to encounter root and crown rot diseases in all development stages of the plant. While most of the disease factors can originate from seeds and soil, their importance increases even more because they cause different diseases in leaves and spikes other than the root and root neck of the plant. For example, *Fusarium* spp. spike blight and *Dreschlera* spp., *Fusarium* spp., *Alternaria* spp. They can also cause embryo darkening (black spot) disease.

Root and crown rot diseases are the leading pathogen groups affecting wheat and barley yield in terms of quality and quantity. In order to develop normally and give the best yield, they must have a healthy root system. Disease agents can cause damage to most of the cereals. These include wheat, barley, triticale, oats and rye. Generally, root and crown rot disease factors in wheat and barley plants reduce the number of plants per square meter, the number of heads per plant, the length of the spikes, and therefore the number of grains per spike. These factors, first of all, are effective in the root and crown parts of the plant, thus reducing the nutrient and water absorption capacity of the plant roots. Root and crown rot disease agents such as *F. culmorum* W.G. Smith, and *G. graminis* var. *tritici* [Sacc.] Arx & Oliver [J. Walker] (*GGT*) cause some spikes to take on a white appearance during the planting period in the fields where the disease is intense. Such spikes cannot form grains or form weak grains. Thus, the disease factors cause damage in terms of quality by reducing the hectoliter weight of the grain. It has been determined by some researchers that the grain weight in the ear decreased by 30-60% due to root and crown diseases, which were quite common in Thrace in 1978, and the 1000-grain weight of the product obtained from the diseased field decreased by 17% compared to the healthy ones. *Fusarium* and *Rhizoctonia* species are the most common and important soil-borne fungi that cause root and root collar diseases in Turkish wheat fields, and the burrowing disease caused by *GGT* has also been a problem in recent years.

GGT, on the other hand, causes the disease known as "take-all" in wheat, barley and triticale. Although its main host is wheat, it causes disease in barley, triticale, rye, other grains and grasses and is considered as one of the most important root diseases affecting wheat yield in many parts of the world (Cook 1992). The fungus can infect plant roots and rot and destroy the entire root system. The root and rhizome turn charcoal black and are easily palpable when the plant is pulled. The fungus invades the stem upwards, causing the plant to collapse. Pathogen lives in the infected root and stem of the plant. Infection persists throughout the growing season. The optimum soil temperature for the disease is 10-20 °C. The disease-causing fungus grows well in neutral to alkaline soils, sloping lands, poorly drained fields, and soils poor in nitrogen and phosphorus (Wiese 1998). It was later reported that it was detected in Sakarya (Aktaş et al. 1996). With the climate change in recent years and the planting of susceptible varieties, the disease has spread to the Central Anatolia Region (Aktaş et al. 1999, Büyük et al. 2018, Tunalı et al. 2008) and Thrace (Hekimhan 2010) and has created serious problems.

Chemical control of *GGT* in the world is limited and difficult. Currently, there is no licensed plant protection product against the disease in Türkiye. It has been reported that the disease causes more than 40% product loss in wheat if it is not controlled (Genowati 2001). Due to the limited chemical control and the harmful effects of chemicals, alternative methods of combating this disease have gained importance. One of the most important of these methods is the use of disease-resistant varieties and alternative methods like biological control.

The inhibition and effects of *Trichoderma harzianum* isolates against wheat root and crown pathogens (*F. culmorum*, *F. pseudograminearum*, *Bipolaris sorokiniana* and *R. solani*) were investigated in sterile and natural soil conditions in 2006. In the inhibition trial, T10 isolate against *F. culmorum* showed 82.6% inhibition*, F. pseudograminearum* T7 72.2%, *B. sorokiniana* T7 76.4%, *R. solani* T1 67.8% inhibition. *T. harzianum* isolates were applied to the wheat seed of Kınacı 97 variety as a coating. In sterile soil, T10 was 65.6% effective against *F. culmorum*, T4 51.9% against *F. pseudograminearum*, T6 63.5% against *B. sorokiniana*, T5 73% against *R. solani*, and against *F. culmorum* in natural soil. T3 reported that it had 23.3%, *F. pseudograminearum* T6 15.8%, *B. sorokiniana* 60.4% T6, and *R. solani* 26.8% T10(9 (Erdurmuş and Katırcıoğlu 2008).

Karahan et al. (2011) obtained 105 non-pathogenic fluorescent *Pseudomonas* isolates from Ankara, Eskişehir and Sakarya provinces in 2007-2009, in *in vitro* tests, they measured the inhibition zones created by antagonistic bacteria against the pathogen *F. culmorum* in petri dishes containing Potato Dextrose Agar (PDA). between), 67 of them were identified as weak antagonist (zone of inhibition <5 mm). Three of the antagonist bacterial isolates were *Pseudomonas fluorescens*, one was *P. putida*, and two were *Pseudomonas* spp. are reported to have been diagnosed. In *in vivo* trials, it has been reported that none of the bacterial isolates showed antagonistic activity against the pathogen *F. culmorum*. Using PGPR and bio agent bacteria to control *B. sorokiniana* and its effect on plant growth were determined and 39 antagonist bacterial isolates, selected considering their antifungal properties, nitrogen fixation and phosphate dissolution properties, were developed in liquid carrier formulation, then tested in terms of its effectiveness on disease development and growth parameters of wheat plants in pot trials using seed coating method. It has been determined that many of the antagonistic bacteria were both effective in suppressing the disease and making important contributions to plant growth. It was concluded that some of the liquid formulations (*B. megaterium* TV-6D, *Brevibacillus choshinensis* TV-53D and *B. pumilus* TV-3C) formed from antagonist bacterial isolates can be used both as bio pesticides in the control of wheat root rot disease and as microbial fertilizer in wheat cultivation (Gökç 2013).

Methods

The fungi *F. culmorum*, *GGT* and *D. sorokiniana* used in this study were provided by the Cultural Collection of Plant Pathogens from Plant Protection Central Research Institute/Ankara, the antagonistic bacterial isolates were supplied from the previous studies conducted by Dr. Kamil DUMAN essentially isolated from soil, seed and plant tissue samples.

Determination of Antagonistic Effects of Isolated Antagonist Bacteria (AntB) Isolates on Mycelial Growth of Fungal Agents: The potential of selected candidate BCA bacterial isolates to inhibit (antagonize) mycelial growth of disease agents was determined by double culture tests in petri dishes containing PDA, as previously reported (Soylu et al., 2005). In these tests, the bacterial isolate to be tested is drawn on the tip of each petri dish, and after pre-incubation at 26°C for 24 hours, 5 mm diameter mycelial discs taken from the growth areas of fresh pathogenic fungus cultures developed in PDA medium were formed so that they were equidistant from the candidate antagonist bacterial isolates developed in the medium placed in the middle and incubated at 26°C. No antagonist bacterial isolate was drawn into the control dishes. Upon reaching the 75% in the control plates, the fungal mycelium growth (MGT) directed towards the bacteria was measured (3-7 days after inoculation depending on the development of the fungal agents) and the % inhibition rates according to the mycelial growth (MGC) in the control plates were calculated using the formula given below calculated accordingly.

$$\%Inhibition = ((MGC-MGT)/MGC) *100$$

For each bacteria-fungi combination, measurements were made in 3 different petri dishes, and the experiment was repeated at 2 different times.

Experimental Design and Statistical Analysis: All trials were set up according to the randomized blocks trial design, without converting the inhibition rates of pathogen development to %, using the SPSS statistical program (SPSS Statistics 17.0), Variance Analysis was performed with one-way ANOVA and the difference between isolates was determined by Duncan's Multiple Range Test. (p≤0.05).

Results and discussion

In *in vitro* efficacy studies, especially with *Bacillus* spp., it is thought that these isolates can be directly converted into a tool (biological preparation) that can be used to prevent the losses caused by disease agents in different crops.

Among 141 different candidate antagonist bacterial isolates 27 of them showed significant inhibition performance which is more than 50% and caused mycelial destruction. 114 of them showed less inhibition but the most important factor that caused the separation of the 27 significantly successful bacterial isolate was they sustained the rate of inhibition even after 21 days after dual culture assay. The rest of the candidate bacterial isolates couldn't sustain the inhibition zone; after 7-14 days the inhibition zone has covered by the pathogen fungi.

When the effectiveness of the tested candidate antagonists on the inhibition of mycelial growth of *F. culmorum* was examined, AntB5 isolate was the most effective isolate with 96.1% inhibition rate, which was followed by AntB18 isolate with a 94.9% inhibition rate, the licensed plant protection product based on *B. subtilis* as a comparative application was placed after these two isolates and caused 94% inhibition. The candidate antagonist isolate that inhibited *F. culmorum* mycelial growth at the lowest rate was AntB15 isolate with a rate of 56.9% (Table 1).

When the effectiveness of the tested candidate antagonists on the inhibition of mycelial growth of *GGT* was examined, AntB5 isolate was the most effective isolate with 100% inhibition rate, followed by AntB7 isolate with a 99.7% inhibition rate, the licensed plant protection product activated by *B. subtilis* showed 100% inhibition. The AntB15 isolate showed the lowest rate with a rate of 49.7% (Table 1).

The isolate AntB5 showed the most efficacy on the inhibition of mycelial growth of *D. sorokiniana* with 89.9% inhibition rate, followed by AntB8 isolate with a 89.8% inhibition rate, the licensed plant protection product with *B. subtilis* basis was placed in third group caused 84.4% inhibition. The isolate AntB15 had the lowest inhibition by a rate of 53.9% (Table 1).

Mean values followed by different letters within each column were significantly different according to Least Significant Difference (LSD) test (P≤0.05)

Many *Bacillus* spp., besides producing antimicrobial compounds, have the ability to form spores that are resistant to adverse environmental conditions. These spores can be easily converted into biological preparations with appropriate technologies and used in the control of many plant diseases (Emmert and Hendelsmann 1999). By investigating the *in vivo* efficacy of these antagonists against the disease agents they are tested for, it will be possible to select the most suitable isolate for use as a biological preparation. In addition, studies need to be carried out on the determination of the mechanisms used by antagonist bacterial biocontrol agents to prevent disease emergence will further increase the widespread impact of the studies.

Table 1. Inhibition percentage of the antagonist bacterial isolates against *Fusarium culmorum*. *Gaeumannomyces graminis* var. *tritici* and *Drechslera sorokiniana* by comparison on petri dishes by dual culture assay

Isolate	*Fusarium culmorum*	*Gaeumannomyces graminis* var. *tritici*	*Dreschlera sorokiniana*
AntB1	81,2±1,0 ^{AD}	78,1±1,2 ^{CE}	81,2±1,0 ^{AF}
AntB2	90,3±0,5 ^{AB}	**99,0±0,5 ^{A}**	76,9±0,6 ^{AG}
AntB3	**92,5±0,2 ^{AB}**	**98,9±0,3 ^{A}**	81,9±0,7 ^{AF}
AntB4	70,2±2,4 ^{CF}	65,3±2,8 ^{DG}	70,2±2,4 ^{CH}
AntB5	**96,1±0,4 ^{A}**	**100±0,0 ^{A}**	**89,9±1,0 ^{A}**
AntB7	92,1±0,1 ^{AB}	**99,7±0,3 ^{A}**	80,4±0,6 ^{AF}
AntB8	89,8±0,2 ^{AB}	88,1±0,3 ^{AC}	89,8±0,2 ^{A}
AntB9	62,4±8,1 ^{EF}	56,1±9,4 ^{FG}	62,4±8,1 ^{GH}
AntB10	91,8±1,6 ^{AB}	**98,2±0,5 ^{AB}**	81,7±3,3 ^{AF}
AntB11	83,6±1,5 ^{AC}	80,8±1,7 ^{BD}	83,6±1,5 ^{AE}
AntB12	63,1±0,6 ^{EF}	56,9±0,7 ^{FG}	63,1±0,6 ^{GH}
AntB13	62,6±5,2 ^{EF}	56,4±6,1 ^{FG}	62,6±5,2 ^{GH}
AntB14	**93,1±0,4 ^{AB}**	**98,6±0,6 ^{A}**	83,7±0,6 ^{AD}
AntB15	56,9±2,7 ^{F}	49,7±3,1 ^{G}	53,9±2,1 ^{H}
AntB16	72,4±0,6 ^{CE}	67,8±0,7 ^{DF}	72,4±0,6 ^{BH}
AntB17	67,9±3,4 ^{DF}	62,5±3,9 ^{EG}	67,9±3,4 ^{EH}
AntB18	**94,9±0,7 ^{AB}**	99,5±0,3 ^{A}	87,8±1,7 ^{AB}
AntB19	67,1±4,9 ^{DF}	61,7±5,7 ^{EG}	67,1±4,9 ^{FH}
AntB20	92,6±0,4 ^{AB}	**98,9±0,3 ^{A}**	82,2±1,3 ^{AF}
AntB21	68,6±3,4 ^{CF}	63,3±3,9 ^{EG}	68,6±3,4 ^{DH}
AntB22	70,7±6,0 ^{CF}	65,8±7,0 ^{DG}	70,7±6,0 ^{CH}
AntB23	80,5±2,0 ^{BD}	77,2±2,4 ^{CE}	80,5±2,0 ^{AF}
AntB24	88,8±0,9 ^{AB}	86,9±1,0 ^{AC}	**88,8±0,9 ^{A}**
AntB25	91,1±1,0 ^{AB}	97,8±0,7 ^{AB}	79,7±1,9 ^{AF}
AntB26	90,1±0,8 ^{AB}	98,2±0,3 ^{AB}	77,6±1,7 ^{AG}
AntBKU	94,0±0,1 ^{AB}	100±0,0 ^{A}	84,4±0,4 ^{AC}
Control	0±0,0 ^{G}	0±0,0 ^{H}	0±0,0 ^{I}

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The impact of plant and fungal based compounds as promising bionematicides on cereal cyst nematode, *Heterodera filipjevi* under *in vitro* and *in vivo* conditions

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Summary

The effect of four promising non-chemical compounds, two plant-based namely Nemakob and Nemakob Plus, in the amount of 4000 ppm, two fungal-based SP2 and CP1, with dose of 5000 ppm, and 6000 ppm of a bacterial-based nematicide Abamectin® were tested on cereal cyst nematode *Heterodera filipjevi* under *in vitro* and *in vivo* conditions. ELISA plates were used for the *in vitro* conditions which contained 120 fresh second stage juveniles (J2) where 150µl of compounds with defined concentrations were added to each well. After 48 hours, the percentage of mortality of J2 was measured. In *in vivo* conditions, the germinated seeds of wheat susceptible cultivar Bezostaya were planted in plastic tubes filled with sterilized soil. One day after planting, each plant was received 500 fresh J2 in one ml of tap water. The compounds were then added to each seedling after 24 hours of nematode inoculation. The plants were maintained at 23 ± 2 °C and harvested after nine weeks and the roots and soil were examined for the development of white females. All data were subjected to analysis using the Generalized Linear Model (GLM) in statistical software SAS v9.4. Comparison of means was performed by Duncan's Multiple Range Test at $p \leq 0.05$. The results showed the most effective treatments on the mortality percentage of J2 were Nemakob, Nemakob Plus and Abamectin®, by 65, 65 and 62.9% respectively in *in vitro*. Whilst the reduction of white females by 100% was revealed by application of Nemakob at concentration of 4000ppm in pot trials. The potential of Nemakob and Nemakob plus, the two plant-based compounds tested in this research, by causing J2 mortality and preventing the formation of female on the root of susceptible wheat cultivar indicates the necessity of further research on these compounds as promising bionematicides.

Introduction

Heterodera filipjevi (Madzhidov) Stelter is one of the main six species parasitizing the cereal crops under the common name of Cereal Cyst Nematode (CCN.) Host plants of *H. filipjevi* include wheat, rye, barley, corn, and many grass species. Given the high economic impact caused by *H. filipjevi*, this species could reduce wheat yields by 42 to 45 % in Türkiye (Nicol *et al.* 2009). Yield reductions caused by *H. filipjevi* in three winter wheat cultivars in Iran ranged from 20.4 to 24.8% (Karimipour Fard et al. 2018).

A large number of strategies have been applied for cereal cyst nematode control in the world including chemical nematicides. But on the other hand, it may cause environmental hazardous and degradation in soil fertility. The development of natural organic and biological nematicides with plant-based, fungus-based and bacterium based components that could be effective in plant parasitic nematodes management and environmentally friendly could be a beneficial method for CCNs control and a safe alternative to chemical nematicides. There are examples of successful application of some bionematicides include the formulation of biological active components such as *Purpureocillium lilacinus* products BioAct WP, BioAct WG, MeloCon, NemOut, *Bacillus firmus* I-1582, Nortica 5WG or *Serratia marcescens* product Namaless against a range of plant parasitic nematodes on important crops (Wilson and Jackson 2013, Crow 2014 and Abd-Elgawad and Askary 2018). Wood vinegar is a liquid substance produced during the production of charcoal (Cai et al. 2012). The effective role of wood vinegar has been proven in organic agriculture and on a number of nematode groups (Mu 2003; Koc 2019). Therefore, the present experiments were conducted to study the effect of two formulations of antagonistic fungus isolates of nematode eggs *Pochonia chlamydosporia* var *chlamydosporia* namely CP1 and SP2, Abamectin®, trade formulation of avermectin of *Streptomyces avermitilis* and two new formulations of wood-vinegar-based nematicides namely Nemakob and Nemakob plus against cereal cyst nematode, *H. filipjevi* infecting wheat crops.

Methods

Nematode inoculum was collected from a naturally infested wheat field with a long history of infestation to *H. filipjevi* in Hamadan province (35.002306N, 48.131956E), then morphological and molecular identification confirmed the isolated nematode species. The nematicidal potential of four compounds including CP1 and SP2 with 5000ppm of 2 x 10^{12} Conidia/Kg, Abamectin®, 6000ppm, Nemakob and Nemakob plus ,40000ppm, and the control with nematodes and without nematicide treatment were tested on J2s of *H. filipjevi* in *in vitro* and *in vivo* conditions. Five replications were considered for each treatment.

In *in vitro* experiment for each treatment 150µl were added before transferring 120 J2s suspended in one ml of tap water to each well in ELISA plates 12*8. Distilled water served as the control. After 48 h of incubation at 23 ± 2℃ juvenile mortality was counted under a stereomicroscope.

In *in vivo* study, germinated seed of Bezostaya as a susceptible cultivar was individually planted into a plastic tube (3 x13 cm) containing sterilized mixture of sand, field soil and organic matter in a ratio of (70:29:1, v:v:v). Then the tubes were arranged in a stand and placed in a white iron tray. A completely randomized design was followed for implementing the experiment containing three replications for each treatment. One day after planting, each plant was inoculated with 500 J2 in 1 ml tap water. Each compound was added to each tube after 24 hours of nematode inoculation. Plants were maintained in growth chamber conditions at 23 ± 2°C, 16 h of artificial light and 65% relative humidity. The plants were harvested after nine weeks and the number of white females on roots were counted. The data were subjected to analysis using the Generalized Linear Model (GLM) in statistical software SAS v9.4. Normalization of phenotypic data was performed using $\sqrt{x+1}$ formula. Comparison of means was performed by Duncan's Multiple Range Test at $p \leq 0.05$.

Results and discussion

In this experiment among the promising non-chemical nematicides tested against *H. filipjevi* under *in vivo* and *in vitro* conditions, three had fungal and bacterial based, *i.e.* CP1, SP2, and abamectin®, and two were plant-based compounds, namely Nemakob and Nemakob Plus. Based on the comparison of the mean percentage of mortality in *in vitro* condition, the best effect on the mortality percentage of the second stage juveniles (J2), were observed in Nemakob, Nemakob Plus and Abamectin®, respectively.

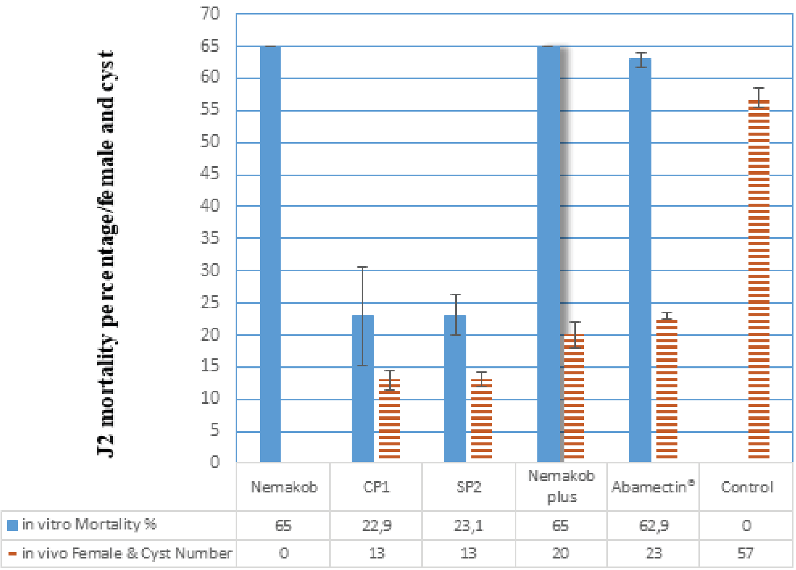
The percentage of mortality of second stage juveniles compared to control were in Nemakob and Nemakob plus with 64.96% and in Abamectin® with 62.9%. Two compounds CP1 and SP2 increased the mortality rate of J2 and decreased the white females and cysts numbers almost to the same extent. The mortality rate of nematode juveniles in both treatments was around 23%. *In vivo* conditions indicated the most effectiveness belonged to plant-based nematicide Nemakob by 100% reduction of white females and cysts compared to the control. The decrease in the number of white females and cysts in CP1 and SP2 promising nematicide compounds compared to the control is more than 77%. The reduction in the number of white females and cysts in Nemakob Plus and Abamectin® treatments compared to the control was 64.9% and 59.7%, respectively. (Figure 1).

Non-chemical compounds with many biological bases have been used to control the cyst nematode *H. filipjevi*. A variety of microorganisms, such as fungi, bacteria, and actinomycetes have been successfully used as biocontrol agents of nematodes on different crops. Bacteria and their metabolites affect both plant and microbial community. Direct antagonistic effect can be achieved by parasitism, antibiosis, or competition for nutrients or infection sites. Indirectly, bacteria can enhance host defense mechanisms provoking induced systemic resistance (Migunova and Sasanelli 2021).

Nematicidal activity against the second stage juveniles (J2) of *Meloidogyne javanica* and *H. filipjevi* was demonstrated in *in vitro* by cultural filtrates of *Bacillus subtilis* OKB105 (100%) (Migunova and Sasanelli 2021). *Bacillus cereus* 09B18 showed 76% and 44% reduction of *H. filipjevi* white females per plant in greenhouse and field conditions, respectively, which were not significantly different from those obtained by Avermectin treatments *(*Migunova and Sasanelli 2021).

Two bacterial strains, *Achromobacter xylosoxidans* (09X01) and *B. cereus* (09B18), were isolated from cereal cyst nematodes (*H. filipjevi*) in Henan, China. The two bacterial strains were subsequently evaluated for their biological control potential. The results showed that the culture filtrate of the two strains caused high mortality of the second stage juvenile (J2) and reduced egg hatching in *in vitro* (Zhang 2016). Many studies have also been reported the use of *Trichoderma* spp. to control cyst nematodes such as *H. avenae* and *H. filipjevi* (Zhang 2016).

Among the plant extracted products, tar and vinegar are used as pesticides and biocides for plant preservative and use of wood vinegar (WV) or pyroligneous acid (PA) increased rapidly in Asian countries including China, Japan, India and Thailand. Due to energetic development of biopesticides, numerous botanical pesticides have an active place in the market since the last decade (Ahmed *et al.* 2022). A large number of papers described the undeniable efficiency of WA for pest control in pure form or combined with conventional pesticides. The nematicidal action of the WA was demonstrated in the management of root-knot nematode (*M. incognita*) in lettuce culture, in greenhouse conditions. The 0.5% concentration of PA contributed with a better effect in reducing *M. incognita* parasitism (Pereira et al. 2020). Meanwhile the benefits of WA application as a nematicide against *Scutellonema bradys* at different concentrations was also studied (De Farias *et al.* 2020).



	Nemakob	CP1	SP2	Nemakob plus	Abamectin®	Control
🟦 in vitro Mortality %	65	22,9	23,1	65	62,9	0
▬ in vivo Female & Cyst Number	0	13	13	20	23	57

Figure 1. Effect of the promising bionematicides on percentage mortality of second stage juvenile of *Heterodera filipjevi* under *in vitro* conditions and the number of white female and cyst in pot trials.

Since wood vinegar obtained from different plant sources contain different substances and properties, so their effect on plant parasitic nematodes is likely different. In current study Nemakob and Nemakob Plus as promising nematicide compounds, were qualified by many investigations and tests of plant sources and special conditions of pyrolysis such as temperature, pressure and different duration of wood vinegar extraction. In both *in vitro* and *in vivo* conditions, mortality of second stage juveniles and reduction of white female and cysts of *H. filipjevi* resulted from the application of these two compounds as promising nematicides. The potential of Nemakob and Nemakob plus, the two plant-based compounds tested in this research, by causing J2 mortality and preventing the formation of female on the root of susceptible wheat cultivar indicates the necessity of further research on these promising bionematicides.

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The effectiveness of *Trichoderma* spp. against the important causal agents of corn root rot diseases in *in vitro* conditions

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Summary

The aim of this study was to determine the antagonistic effects of three different *Trichoderma species, T. atroviride*, *T. harzianum* and *T. afroharzianum* on four species of *Fusarium species F. pseudograminearum*, *F. verticilloides*, *F. subglutinans* and *F. proliferatum*. In this study, firstly the pathogenicity of *Fusarium species* was tested on susceptible corn variety in petri dishes and disease severity was calculated 64%, 61%, 65% and 59% for *F. verticilloides*, *F. subglutinans*, *F. proliferatum* and *F. pseudograminearum* 59% respectively. Then, the interaction of *Fusarium* and *Trichoderma* was examined. *Trichoderma* spp isolates and pathogenic fungi were grown in PDA as a double culture with 5 replicates. After 7 days, the growth diameters of the antagonist and pathogens were measured and the inhibition rate was calculated. The maximum inhibition (31%) was observed between *F. pseudograminearum* and *T. atroviride*, while the lowest inhibition (61%) was observed between *F. verticilloides* and *T. afroharzianum*. While the most effective *Trichoderma species* against *Fusarium species* was *T. atroviride*, the least effective *Trichoderma species* was *T. afroharzianum*. As a result, it was observed that 3 different *Trichoderma species* significantly affected the development of 4 different *Fusarium species*. These results support the view that *Trichoderma species* could be used as a potential agent to inhibit the growth of pathogens.

Introduction

Chemical control is widely used for controlling of plant diseases. It is known that pesticides have some negative effects on the environment and consumers. The disadvantages of chemical control are costly in terms of economy and pollution the environment with residues. Apart from this, they remove for creation of resistant races with continuous use against target organisms (Delen 1991). For all these reasons, it is necessary to reduce or, if possible, eliminate the use of chemical pesticides in agriculture. This is possible with biological control agents (BCA). The use of BCA in the control of plant diseases is to minimize the effects of chemicals on the environment (Bora and Özaktan 1998). Recently, many BCAs have been licensed as commercial products and are used in agriculture. Microbial biological control agents can be listed as biopesticides, biofertilizers, growth promoters and natural immunity stimulators. *Trichoderma*-based preparations are used in the plant protection struggle against some plant pathogens and to increase plant growth or productivity.

Corn fungal diseases can be grouped according to the damage they cause to the vegetative organs of the corn. The most important disease factors limiting yield and seen in corn fields in Türkiye are; *Fusarium species* that cause seed, root, stem, ear and grain rot. In a study conducted in Türkiye, cereal root and root rot pathogens were identified as *F. culmorum* (14%) *B. sorokiniana* (10%), *Fusarium pseudograminearum* (2%), *Gaeumannomyces graminis* (2%), *Pythium* spp. (3%) *Rhizoctonia* spp. (22%) were detected at different rates (Tunalı et al. 2008). Root rot can result in crop loss of 50% or more where disease propagation is appropriate (Wallwork 2000).

The use of microorganisms in the control of pests in agriculture is one of the most effective strategies of biological control. The potential of *Trichoderma species* to be a biological control agent has been known since the 1930s (Harman 2006). *Trichoderma* interacts with other microorganisms, but especially with pathogenic fungi. These interactions include hyperparasitism, antibiosis, and competition (Küçük and Kıvanç 2004). *Trichoderma harzianum* isolates are used as an alternative to chemicals against many fungal plant pathogens that cause disease in agricultural products (Vinale et al. 2008). *Trichoderma harzianum*, which is in this group, is a commonly used agent in biological control (Elad et al.1984, Sivan and Chet 1986, Bora and Özaktan 1998, Küçük and Kıvanç 2003). In a study conducted in Türkiye, in inhibition experiments of *T. harzianum* isolates, were effective on *F. oxysporum, F. culmorum, F. moniliforme, R. solani, R. cerealis, S. rolfsii, B. sorokiniana, G. graminis* var. *tritici* (Küçük and Kıvanç 2001).

The aim of this study was to determine the antagonistic effect of three different *Trichoderma species* (*Trichoderma atroviride, T. harzianum, T. afroharzianum*) against four *Fusarium species* (*F. pseudograminearum, F. verticilloides, F. subglutinans* and *F. proliferatum*) in laboratory conditions.

Methods

Plant Material

The main material of our study was the variety of sweet corn (*Zea mays* convar. saccharata var. rugosa) obtained from Sakarya Maize Research Institute Directorate.

Culture of Fungus Isolates

The species of *Fusarium* namely *F. proliferatum, F. subglutinans, F. pseudograminearum, F. verticilloides,* and *Trichoderma species* i.e. *Trichoderma harzianum, T. afroharzianum* and *T. atroviride* were provided from Central Research Institute of Plant Protection, Department of Plant Diseases, Cereal Diseases Unit Culture Collection. The isolates were stored at -20°C.

Inoculation for Interaction of Fusarium spp and Trichoderma spp.

The *Fusarium species* were grown on Potato Dextrose Agar (PDA). Five mm fungus plugs were prepared with a cork borer from PDA plates, *Trichoderma* spp isolates and pathogenic fungi were cultured in PDA as a double culture with 5 replicates. Then fungal plugs with a diameter of 5 mm in sterile plates with a diameter of 9 cm containing PDA were cultured at 24 °C for 7 days, with a distance of 5 cm between them. The growth diameters of the antagonists and pathogens were measured and the inhibition rate was calculated by the formula (RI)=(R1- R2)x 100/R1 (RI: Inhibition ratio, R1: Growth radius of the pathogen, R2: Growth radius in the antagonist direction of the pathogen) (Royse and Ries 1978).

Pathogenicity Test

For the pathogenicity test, *Fusarium species* were grown purely on PDA at 24 °C under 15 hours of light and 9 hours of dark conditions. Seeds of the sensitive corn variety used in the experiment were soaked in 1% NaOCl for 1 minutes. After surface disinfection, seeds were germinated for a week. After germination five *Fusarium* plugs (one plug per plate) were placed 1 cm apart from the plantlets. Plates were sealed with parafilm. A single fungal plug and germinated seed were placed in each plate. The procedure was run as 5 replications and 1 control. Only germinated seeds were used as control. Plates were kept at 25°C for 7 days and at the end of the 7th day, pathogenicity was evaluated using scale 0-5 scale, 0: No signs of diseases, 1: 10% of the hypocotyl is infected, 2: 11-30% of hypocotyls infected, 3: 31-50% of hypocotyls infected, 4: 51-80% of the hypocotyl infected, 5: All hypocotyls infected (Ichielevich-Auster et al., 1985).

The results obtained from all pathogenicity tests were calculated according to the formula given below and % disease severity grades were found for each isolate:

Disease Severity (%) = $\Sigma(n x V / Z x N) x 100$, n: the number of plants with different disease degrees on the scale, V: scale value, Z: highest scale value, N: total number of plants observed

Results and discussion

The pathogenicity tests of *Fusarium species* on germinated seeds of corn showed the disease severity was 64%, 61%, 65% and 59% for *F. verticilloides, F. subglutinans, F. proliferatum* and *F. pseudograminearum* 59% respectively in petri dishes conditions.

Results from this study revealed that at the end of the 7th day, the maximum inhibition (31%) was observed in the interaction between *T. atroviride* and *F. pseudograminearum*, while the lowest inhibition (61%) was observed in the interaction between *F. verticilloides* and *T. afroharzianum*. Moreover, while the most effective *Trichoderma species* against *Fusarium species* was *T. atroviride*, the least effective *Trichoderma species* was *T. afroharzianum*. Similar findings were observed by Erdurmuş and Katırcıoğlu (2008). They investigated the inhibitory effect of *T. harzianum* isolates against wheat root and root collar pathogens (*F. culmorum, F. pseudograminearum*, *Bipolaris sorokiniana* and *Rhizoctonia solani* and found that different isolates were effective against different pathogens.

Antibiosis, mycoparasitism and enzymes produced by fungi are thought to play a role in the mechanism of action of *T. harzianum* isolate used in *in vitro* studies. Indeed, Monaco et al. (2004) and Küçük and Kıvanç (2003) found similar results and reported that the metabolites produced by *T. harzianum* have an inhibitory effect on the growth of pathogens. In this study, it was observed that the effects of *T. harzianum* on the development of different *Fusarium species* may be different. Fungi growth was observed in *F. proliferatum* at 51%, *F.subglutinans* at 67% and *F. pseudograminearum* at 48%. Similar findings have been reported, for example, by Küçük and Kıvanç (2001) who found that *T. harzianum* isolates were effective against *F. culmorum* with 77% inhibition. *T. harzianum* isolates tested in another study were found to inhibit the growth of *F. verticilloides* (Cevheri and Küçük 2012).

In the *T. arthroviride* interaction study, fungal growth was observed in *F. proliferatum* at 45%, *F. subglutinans* at 54% and *F. pseudograminearum* at 29% (Table 1). As can be seen in table 1, while the most effective inhibition was observed in *F. pseudograminearum*, the least inhibition was observed in *F. subglutinans*. Moreover, in the interaction between *T. afroharzianum* and *Fusarium* spp, the inhibition rates were found at an average of 60% in the three *Fusarium species* (*F. proliferatum* 61%, *F. subglutinans* 66%, *F. verticilloides* 69% and *F. pseudograminearum* 46% fungus growth).

Table 1: Growth of *Fusarium species* under the inhibition by *Trichoderma species* in laboratory conditions

Causal Agent	*F. subglutinans*	*F. pseudograminearum*	*F. proliferatum*	*F. verticilloides*
T. harzianum	67,79%	48,33%	51,667%	68,08%
T. artroviride	54,8%	29,16%	45%	60,63%
T. afroharzianum	66,66%	46,66%	61,66%,	69,14%

In conclusion, in this study, it was observed that 3 different *Trichoderma species* significantly affected the growth of 4 different *Fusarium species*. These results supported the view that *Trichoderma* spp. could be used as a potential agent to inhibit the growth of pathogens. However, how *Trichoderma species* can increase their activity in the soil and the mechanisms of protection against future soil-borne pathogens still need to be investigated further.

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The effects of wheat-vetch rotation and different tillage methods on the population development of root lesion nematodes (*Pratylenchus* spp.)

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Summary

Wheat cultivated areas in Türkiye was recorded as 6.7446 million hectares based on data in 2021. Yield production from these areas was about 17.65 million tons. Also in Türkiye 0.3653 million hectares of vetch cultivated areas were recorded in 2021. Having wheat and vetch as their important role in Türkiye's agricultural production, it also increases the importance of pest control of related crops. Plant parasitic nematodes have a potential to cause significant yield losses among pests. In this study, the population development of root lesion nematodes (*Pratylenchus* spp.) was determined in wheat and vetch cultivated areas in Çanakkale Province, Dardanos Campus. The effects of crop rotation and different tillage methods on root lesion nematode populations were investigated during 2019-2021. Wheat-vetch crop rotation was applied in the area where the study was conducted and 3 different tillage equipments were used. In the area of six plots, the highest nematode density was observed in the Chisel plow-Wheat plot and the lowest was in Plow-Vetch parcel. The density of *Pratylenchus* spp. decreased during crop rotation period. The use of different tillage equipment, had a direct effect on the root lesion nematodes. densities.

Introduction

Wheat is one of the most important plants which provides a basic food source for humans. Parallel to the increase in world population, the need for wheat also increases. It is estimated that the world population, which was 6.1 billion in 2000, will reach 9.3 billion in 2050 (Anonymous 2009). As a result of this increase, the world's wheat demand is expected to increase in parallel. Wheat is one of the most important grains in terms of nutrition in Türkiye. In 2021, the wheat planted area in Türkiye is 6.7 million 446 thousand hectares. The crop obtained from these areas is 17 million 650 thousand tons (TÜİK 2021). Vetch, which is used as animal feed, has an important place in agriculture and animal husbandry in our country. Animal foods are a very effective and important area in the nutrition of human beings. The way to get enough animal food is to increase animal products, the way to increase animal products is to feed animals with abundant and high-quality roughage and improve domestic animal breeds (Anonymous 2010). Vetch, which is an important agricultural product, is used in two different ways as green grass and dry grass. In Türkiye, vetch was planted on an area of 0.3 million 653 thousand hectares in 2021. 3 million 990 thousand tons of green grass production were obtained from these areas (Tuik 2021).

The fact that wheat and the vetch plant have an important place increases the importance of pest control. Nematodes have a critical place among these damages in terms of the damage they cause. It is estimated that plant parasitic nematodes cause an average of 7% product loss in wheat worldwide, and its financial value is reported to be 5.8 billion dollars (Sasser 1987). The nematode species determined to cause economic losses in wheat cultivated areas around the world; Cereal cyst nematodes (*Heterodera* spp.), root lesion nematodes (*Pratylenchus* spp.), Wheat gall nematode (*Anguina tritici*), Root-knot nematode (*Meloidogyne* spp.) and stem and bulb nematode (*Ditylenchus dipsaci*) have been reported (Nicol et al. 2002). In studies conducted in different regions of Türkiye, it has been determined that cereal cyst nematodes and lesion nematodes are common in wheat production areas and cause significant losses in yield (Yıldırım et al. 2007). In another study, *Pratylenchus* spp. caused a yield loss of 36% in the Eastern Mediterranean Region of Türkiye (Elekçioğlu and Gözel 1997).

It has been determined by studies that a life stage of *Pratylenchus* spp. is completed in 40-45 days at 27°C under laboratory conditions and the adults complete their development in 35-40 days under suitable natural conditions, depending on the type of plant, air temperature, humidity, and other environmental conditions (Nicol 1996). The nematode can feed by entering the roots in all juvenile and adult stages. Root lesion nematodes spend the winter inside infected roots or in the soil as eggs, juvenile, or adults under winter conditions. In dry periods when the temperature and humidity are low, it remains stagnant in the soil until the next plant development period (Agrios 1969).

Pratylenchus spends all its life stages in the root zone (Kepenekçi 2012). Root lesion nematodes, which are polyphagous pests, have a wide host range including barley, wheat, and chickpea (Handoo and Golden 1989; Castillo and Vovlas 2007). Root lesion nematodes, which can reproduce very quickly, migrate between the capillary roots of the plant and may form brown spots on the root part of the plant as a result of feeding on the roots (Agrios 1997).

The most effective control method against root lesion nematodes is the use of resistant varieties. Crop rotation is also an important method of control in terms of pest control. Many studies show that it reduces the damage level of pests and diseases in crop rotation areas. Considering these situations, the importance of combating nematodes increases and makes it necessary. This study was carried out to investigate the responses of root lesion nematodes (*Pratylenchus* spp.) found in wheat and vetch fields in Dardanos Campus of Çanakkale Onsekiz Mart University to wheat-vetch rotation and the effects of different tillage tools on population distribution.

Methods

This study was carried out to determine root lesion nematodes in wheat and vetch fields in the Dardanos Campus of Çanakkale province and to examine the effect of crop rotation and different tillage tools on the population during two production seasons. This study aimed to determine the population of the nematode, the application of crop rotation, and the effect of different tillage tools on the nematode population. For this purpose, the study was carried out in two ways as soil sampling from the determined plots every month and the preparation and examination of the samples taken in the laboratory.

Soil Sampling: The total area of 0.8 hectares where soil samples were taken consisted of 6 equal parcels of approximately 1000 squaremeter. Wheat and vetch crop rotation was applied in the field. In addition, 3 different tillage tools were used. In the order of naming according to the product pattern and the tillage tool; plow-vetch (PF), plow-wheat (PB), rotatile-wheat (RB), rotatile-vetch (RF), Chisel plow-vetch (CF), and Chisel plow-wheat (ÇB) forms. Soil samples were taken from 25 different places of each parcel with the help of a soil auger to represent the parcel homogeneously.

Samples were taken from the trial area in October 2019 and the last sample was taken in September 2021, the sampling process continued in both seasons. Soils taken from 25 different parts of a parcel with a soil auger were mixed in a large diameter plastic container and 2 kg homogeneous soil samples were obtained. A total of 6 different soil samples were taken from the area with 6 parcels, each weighing 2 kg. Soil samples were taken from 0-30 cm depth of the soil where plant parasitic nematodes are concentrated. The samples taken were labelled according to the type of plant in the area, the tillage tool, and the date and put in plastic bags. Soil samples were brought to Çanakkale Onsekiz Mart University Nematology Laboratory in an ice box. The samples brought to the laboratory were stored in climate chambers at +4°C for later analysis.

Laboratory Studies: Nematodes were extraced from the soil by the improved Baermann Funnel Method (Hooper 1986). The recovered nematodes were counted in 100 µl of nematode suspension with the help of a micropipette and examined under a light microscope and the density of root lesion nematodes.were determined in soil sample.

Results and discussion

The population fluctuations of root lesion nematodes (*Pratylenchus* spp.) of wheat-vetch rotation and different tillage methods showed time-dependent changes according to rotation and tillage methods (Figure 1).

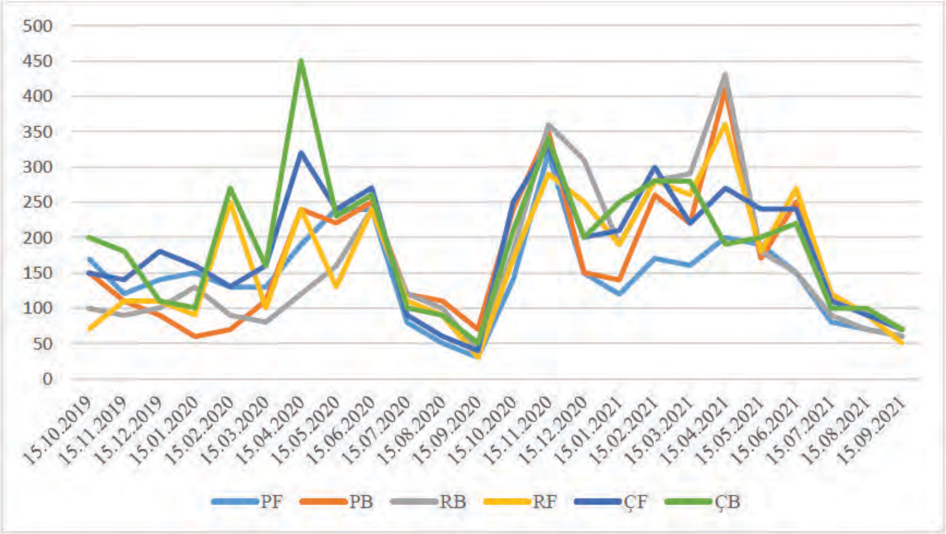

Figure 1. Population fluctuation of *Pratylenchus* spp. in trial plots including plow-vetch (PF), plow-wheat (PB), rotatile-wheat (RB), rotatile-vetch (RF), Chisel plow-vetch (CF), and Chisel plow-wheat (ÇB) forms (Nematode/ 100 g soil).

Field was divided into 6 plots, where crop rotation was applied, 3 types of tillage tools were used. The periods with the highest density of *Pratylenchus* spp. were between March-April-May in both seasons. The periods with the lowest density were determined in the months of July-August-September when there was no vegetation, the temperature was high and there was no precipitation. Also, during the months of November-December-January, *Pratylenchus* spp. the level was detected as low when the precipitation level was very high and the temperature was at a low level. Studies have shown that root lesion nematodes go into a stagnant period in dry periods when the temperature is high. In the study conducted in the Mediterranean region, it was determined that the population density of *Pratylenchus* spp. was high in March, and the population decreased during the periods when the temperature increased (Elekçioğlu and Gözel 1997).

In April, the *Pratylenchus* density in the ÇB plot reached the highest density among the plots. In a similar study conducted in Türkiye; Şahin et al. (2008) investigated the effects of *Heterodera filipjevi*, *P. thornei*, and *P. neglectus* populations against seasonal changes in a wheat field in Haymana district of Ankara. In their research, they reported that the lowest density was observed in *Pratylenchus* species between the snowy and cold months of November-April. Likewise, they stated that there was a significant decrease in the field population in June and July with increasing temperatures. Significant reductions in yield can occur in high root lesion nematode populations in wheat. In another study, in the US states of Oregon and Washington, it was found that if approximately 611.6 *P. neglectus* is found in 100 cm3 of soil, the yield loss in the winter season is 37%, and in the summer season, when 244.65 *P. neglectus* is found in the soil, the yield loss was reported as 14% (Johnson et al. 2008).

The working depth of tillage tools is important for the transportation of nematodes. Differences can be observed in the densities of nematodes in the soil, depending on the working depth of the tillage tools. Wu et al. (2019) followed the population development of *P. neglectus* in winter wheat growing areas (Jimai 22 and Tainong 18 varieties) for two years (2009-2011), in a study they conducted in China. As a result of the study, they found the maximum nematode density to be 464.00 and 326.66 in the 2009-2010 season, and 199.33 and 339.33 in the 2010-2011 season, respectively, in cultivated wheat varieties. In another study on the damage of root lesion nematodes, it was reported that *P. thornei* and *P. neglectus* caused a yield loss of 30-70% in wheat (McDonald and Nicol 2005; Thompson et al. 2008; Vanstone et al. 2008) and they mentioned that the nematode population decreased during the periods of rotation.

In this study, the effect of wheat-vetch crop rotation and three different tillage tools on the population growth of *Pratylenchus* spp. was investigated in 2019-2021. The soil tillage tool with the highest nematode density was the Chisel plow. The parcel with the least nematode population density was determined as the Plow-Vetch parcel. It was observed that the population density was low in the plots cultivated with plows and rotary tillers in both years, even in April when the field population is high. In this study, it was determined that deep tillage tools decrease the current nematode population in the field.

Population densities of nematodes in soil may vary depending on soil temperature and weather conditions. In conditions where soil temperature and humidity are low, nematodes move deeper into the soil, and in conditions where soil temperature and humidity are optimum, they find a habitat at a depth of 0-30 cm in the soil. In the months of March-April-May, the nematode density was found to be at the highest level in conditions with average precipitation and temperature. In the months of July-August-September and December-January-February, the density was determined at the lowest levels in the weather conditions where the temperature is the highest and the lowest. Finally, with this contribution, it has been determined that the use of different tillage tools can change the nematode density in soil and it is revealed the importance of crop rotation in the plant parasitic nematode management in wheat growing areas.

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Nematicidal activity of some essential oils against cereal cyst nematode, *Heterodera filipjevi* in *in vitro*

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Summary

Cereal cyst nematodes (CCN) are a serious limitation to wheat production, causing large crop losses worldwide. CCN species *H. avenae*, *H. filipjevi* and *H. latipons* are the most frequently encountered cyst nematode species attacking cereals, particularly wheat and barley. *Heterodera filipjevi,* the most harmful species especially under drought stress, has a global distribution in wheat and barley growing areas and it has been observed to affect wheat yield. This present study was performed to determine the efficacy of *Artemisia absinthium*, *Tanacetum vulgare*, *Laurus nobilis*, and *Foeniculum vulgare* essential oils against *H. filipjevi* second stage juveniles. Five different concentrations (2, 1, 0.5, 0.250, 0.125 µl/ml^{-1}) of essential oil were used. All treatments were replicated *five* times with two repetitions and sterile water was used as a control. The numbers of immobile nematodes were counted under an inverted microscope after 24, 48, 72 h post incubation. In the experiment essential oil of *T. vulgare*, *L. nobilis*, and *F. vulgare* showed an increasing nematicidal effect according to dose and time. *Tanacetum vulgare* and *L. nobilis* showed 99.7% and 100% effect after 72 hours, respectively, at a dose of 2 µl/ml^{-1}, while *F. vulgare* showed a 100% effect after 48 hours at a concentrations of 0.25 µl/ml^{-1}. As a result of study the essential oil of *T. vulgare*, *L. nobilis*, and *F. vulgare* exhibited potential nematicidal action against *H. filipjevi*. However, pot and field trials should be conducted to observe the actions of these essential oils.

Introduction

Wheat (*Triticum aestivum* L.) is cultivated worldwide and is one of the world's population's primary sources of calories and protein. Wheat is grown in 162 different countries with 760.9 million tons of production and is the second most important staple food after corn in the world and first in Türkiye (FAOSTAT 2020). Wheat production is affected by many pathogens and pests, including nematodes. The damage caused by plant parasitic nematodes is often overlooked because the symptoms of nematodes are often confused with other biotic and abiotic factors. However, there is a 12% loss in total agricultural production in the world caused by plant parasitic nematodes (Stirling et al. 1999). It is reported that plant parasitic nematodes cause an average of 7-10% product loss, at a cost up to $100 billion per annum in wheat production every year (Sasser 1987, Whitehead 1998, Bird and Kaloshian 2003). Damage can reach up to 35-40% depending on biotic and abiotic stress factors such as species, population density, and drought (Williamson and Gleason 2003).

Wheat is associated with numerous plant parasitic nematodes, but only cereal cyst (*Heterodera* spp.), root lesion (*Pratylenchus* spp.), seed gall (*Anguina tritici*), root knot (*Meloidogyne* spp.), and stem and bulb nematode (*Ditylenchus dipsaci*) are considered economically significant (Dababat and Fourie 2018). Cereal cyst nematodes (CCN) are a serious limitation to wheat production, causing large crop losses worldwide. CCN species *H. avenae* (Wollenweber), *H. filipjevi* Madzhidov (Stelter), and *H. latipons* (Franklin) are the most frequently encountered cyst nematode species attacking cereals, particularly wheat and barley (Rivoal and Cook, 1993; McDonald and Nicol, 2005). *Heterodera filipjevi,* the most harmful species especially under drought stress, has a global distribution in wheat and barley growing areas and it has been observed to affect wheat yield in America, Europe, and Asia (İmren et al. 2020, Bishnoi and Bajaj 2002, Smiley et al. 2008). It was determined that *H. filipjevi*, which was determined to cause a 48% loss in 20 eggs and J2/ (g soil)$^{-1}$ density in Iran, caused an average of 42% loss in rain fed winter wheat locations in Türkiye (Hajihasani et al. 2010, Nicol et al. 2004).

Plant parasitic nematodes management includes cultural, physical, chemical and biological methods. The use of resistant varieties is one of the most practical and economical methods of controlling CCN. However, the biggest adversity is that limited number of cultivars has resistance and the emergence of populations that break the resistance. Moreover; intensive use of chemicals, in addition to the impact on non-target organisms, leads to increased production costs and serious health problems for farmers and consumers. For these reasons, A.B. (MBTOC 2006; EC Directive 1107/2009) and as a result of the changes made in Türkiye's legislation, the use of many fumigant and non-fumigant nematicides such as 1,3-Dichloropropene, Aldicarp, Carbofuran, Cadusafos, Ethoprophos, which are widely used in the market, is prohibited (Anonymous 2020). Therefore, developing alternatives to chemical nematicides, which are extremely toxic and dangerous for the environment, is one of the most important priorities for the future of the fight against plant parasitic nematodes (Barker et al. 1994, Dababat and Fourie 2018).

The objective of this study was to determine the nematicidal effect of 10 plant essential oils at five different concentrations and at three different application time intervals on the second stage juveniles of cereal cyst nematode *H. filipjevi*.

Methods

Plant material and essential oils

Artemisia absinthium (Asteraceae), *Tanacetum vulgare* (Asteraceae), *Laurus nobilis* (Lauraceae), and *Foeniculum vulgare* (Apiaceae) were used in the study. Essential oils were obtained as a result of hydrodistillation of the dried and finely divided aerial parts (100 g) of the plant in a Clevenger apparatus for 3 hours. The isolated essential oil was purified from the water it contained on Na_2SO_4 and oils were kept in amber vials at -20°C.

Nematodes

CCNs were collected from the soil of severely infested wheat fields in Kadınhanı in Konya province in Türkiye, at the end of the wheat growing season. Cysts were extracted from the soil via Fenwick can (EPPO 2013).The cysts were incubated at 4°C for 60 d, then at 16°C to stimulate the hatching of pre-parasitic second-stage juveniles (J2s) (Cui et al. 2015).

Bioassasys

Stock solutions of essential oils were prepared at 200µl/ml in methanol (MERC- CAS No:67-56-1) and 16-well plates were used in the experiments. Second stage juveniles were added to each well in 990 µl (120-140 J2) distilled water and 10 µl of each essential oil was added. Experiments were carried out with 5 replications and 2 repetitions using 2, 1, 0.5, 0.250, 0.125 µl/ml^{-1} concentrations of each essential oils. Plates were sealed with plastic films to prevent evaporation and placed in an incubator in dark conditions at 25°C. Dead and alive nematodes were counted after 24, 48, and 72 hours under an invert microscope (Leica DMI 400B). The J2 were considered dead when they did not move on probing with a fine needle (Andre´s et al. 2012, Cayrol et al. 1989).

Statistical analysis: The mortality data were converted to percent mortality and then transformed by arcsine transformation. One-way analysis of variance was used to test the significance, and treatment means were separated by Tukey's multiple comparison test. The statistical analyses were carried out on MINITAB (Release 18) computer program.

Result and discussion

As a result of the studies in which the nematicidal activity of four different plant essential oils against *H. filipjevi* second stage juvenile under laboratory conditions, it was concluded that the activity changed depending on the application concentration, application time, and essential oil ($P<0.05$) (Table 1). The lowest effect was determined in *A. absinthium* essential oil at all concentrations and times compared to the other three essential oils. In *A. absinthium* essential oil, the difference was determined between the times depending on the concentrations, however, the difference between the concentrations depending on the time began to be determined after 48 hours. The highest effect was detected at the end of 72 hours with 82.1% at the dose of 2 µl ml^{-1}. A difference was observed between concentrations from the 24-hour in essential oils of *T. vulgare*, *L. nobilis*, and *F. vulgare*. In *T. vulgare* essential oil, a 99.7% effect was determined at a dose of 2 µl ml^{-1} after 72 hours. After 48th hours, 90.2% effect was determined at 0.5 µl ml^{-1} concentration, while 83.5% effect was determined at 2 µl ml^{-1} concentration after 24 hours. In *L. nobilis* essential oil, 100% and 99.4 effects were determined in 2 µl ml^{-1} concentration after 72 and 24 hours respectively, and 93.7% effect was determined at 0.5 µl ml^{-1} concentration after 48 hours. In *F. vulgare* essential oil, where the highest effect.

There are many studies on the nematicidal effect of essential oils on plant parasitic nematodes, especially root-knot nematodes (Andrés et al. 2012, Ntalli and Caboni 2012). In many studies on root-knot nematodes, very promising results have been obtained and even EC50 values similar to chemical nematsites have been determined in some essential oils (Onifade et al. 2008, Aissani et al. 2015, Oka 2001). However, there are very few studies on cyst nematodes. There are three studies on *Globodera* spp. and eight studies on *Heterodera* spp. that have been conducted so far regarding the nematicidal effect of essential oils on cyst nematodes. No studies were found about *H. filipjevi*. There is no study on the effect of essential oils on *H. filipjevi*, and also of plants belonging to the Asteraceae and Apiaceae families on *Heterodera* spp. (Faria and Vicente 2021).

The nematicidal effects of some essential oils on *H. filipjevi* were examined in this study. The essential oils of *T. vulgare*, *L. nobilis*, and *F. vulgare* exhibited potential nematicidal action against *H. filipjevi*. However, pot and field trials should be conducted to observe the actions of these essential oils. Furthermore, research on the mechanism of action of essential oils on cereal cyst nematodes is required to determine the biological targets of the different components in order to develop more environmentally friendly pest control strategies.

Table 1. The impact of some essential oils on mortality of second stage juveniles of *Heterodera filipjevi* under in *in vitro* conditions

	Mortality (%)±StDev ***Artemisia absinthium***		
Concentration	24 HAT	48 HAT	72 HAT
0.125 µl ml-1	44.9±0.7aC*	47.3±1.3cB	57.4±1.3cA
0.25 µl ml-1	45.6±1.3aC	52.0±1.1cB	69.7±0.7bcA
0.5 µl ml-1	49.8±1.2aC	57.4±1.3bB	70.7±0.5bA
1 µl ml-1	50.5±1.0aC	57.4±0.3bB	72.0±1.1bA
2 µl ml-1	54.3±0.1aC	69.0±0.3aB	82.1±0.8aA
Control (MeOH)	3.0±0.1bB	3.5±0.2dB	3.9±0.1dA
	F=49.75; df=5.30; P<0.05	F=62.89; df=5.30; P<0.05	F=91.10; df=5.30; P<0.05
	Tanacetum vulgare		
0.125 µl ml-1	48.5±0.4cC	62.4±1.1cB	74.1±1.1dA
0.25 µl ml-1	66.5±0.6bC	71.8±0.2cB	86.2±0.8cA
0.5 µl ml-1	77.7±1.3abB	90.2±0.9bA	94.5±0.7bA
1 µl ml-1	76.5±1.2abB	94.1±0.1abA	96.2±0.2abA
2 µl ml-1	83.5±1.7aB	97.0±1.3aA	99.7±0.8aA
Control (MeOH)	3.3±0.1dB	4.1±0.2dA	4.5±0.1eA
	F=87.24; df=5.30; P<0.05	F=180.10; df=5.30; P<0.05	F=208.01; df=5.30; P<0.05
	Laurus nobilis		
0.125 µl ml-1	47.3±0.5cC	67.3±1.2dB	84.1±0.6dA
0.25 µl ml-1	80.6±0.5bB	86.2±0.3cA	88.1±0.2dA
0.5 µl ml-1	84.9±0.3bB	93.7±0.3bcA	94.2±0.2cA
1 µl ml-1	88.6±0.2bB	98.3±1.1abA	98.5±1.0bA
2 µl ml-1	99.4±0.8aA	99.8±0.5aA	100.0±0.0aA
Control (MeOH)	0.8±0.5dC	2.1±0.1eB	3.0±0.2eA
	F=313.89; df=5.30; P<0.05	F=267.93; df=5.30; P<0.05	F=441.91; df=5.30; P<0.05
	Foeniculum vulgare		
0.125 µl ml-1	40.7±4.0cC	67.4±1.2bB	79.0±0.6bA
0.25 µl ml-1	79.8±1.0bB	100.0±0.0aA	100.0±0.0aA
0.5 µl ml-1	97.8±1.4aB	100.0±0.0aA	100.0±0.0aA
1 µl ml-1	99.3±0.9aB	100.0±0.0aA	100.0±0.0aA
2 µl ml-1	99.9±0.3aA	100.0±0.0aA	100.0±0.0aA
Control (MeOH)	0.0±0.3dB	0.5±0.7cB	1.3±0.9cA
	F=157.96; df=5.30; P<0.05	F=688.69; df=5.30; P<0.05	F=807.62; df=5.30; P<0.05

* Means followed by the different small letters within a column and the different capital letters within a row are significantly different according to TUKEY test at $P < 0.05$ level was observed among essential oils, 97.8%, 99.3%, and 99.9% effects were determined at 0.5, 1, and 2 µl ml^{-1} doses, respectively, after 24 hours. A 100% effect was detected in the same essential oil at a concentration of 0.25 µl ml^{-1} after 48 hours.

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Natural antagonists of *Heterodera avenae* under various conditions of cereal crops in Algeria

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Summary

A study was carried out to determine the diversity of the microflora associated with the cereal cyst nematode, *Heterodera avenae*, in four cereal production regions of Algeria. The isolation was mainly targeted toward fungi associated to cyst nematodes. Our study yielded six fungi: *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Rhizomucor* spp., *Rhizopus* spp. and *Ulocladium* spp.. In all the prospected regions, *Fusarium* spp. is the most frequently associated with cysts. The highest diversity is observed at Dahmouni with the following genera *Aspergillus*, *Rhizomucor*, *Rhizopus* and *Ulocladium*. The in vitro test showed that the metabolites of *Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp. parasitize the eggs at a high rate and thus constitute potential agents for the control of *H. avenae*.

Introduction

Cereal cyst nematode, *Heterodera avenae* Woll., is a sedentary endoparasite of cereal roots. In Algeria, this species was reported by Scotto La Massese in 1961. Subsequently, several studies have shown the presence of *H. avenae*in several cereal regions, namely Dahmouni (Tiaret), Djendel (Ain Defla), Ain Azel (Setif), Oued Fodda (Chlef), Hmadna (Relizane) and Hachimia (Bouira) (Mokabli et al. 2001; Smaha et al. 2014; Tirchi et al. 2016; Smaha and Mokabli 2017; Djetti et al. 2018). Crop rotation and the use of resistant varieties are the main measures for controlling this genus. Other alternatives, such as biological control, should be examined with the natural antagonists of this nematode. The first evidence of the natural regulation of *H. avenae* populations was made on cereal monocultures in England (Collingwood 1962) where this phenomenon is widespread as in other countries of northern Europe (Kerry et al. 1982; Stirling 1991). The most important parasitic fungi of *H. avenae* are *Nematophthora gynophila* Kerry and Crump and *Pochonia chlamidosporia* (Goddard) Zare, Gams and Evans (=*Verticillium chlamydosporium* Goddard) (Kerry et al. 1982). In Peru, *Meloidogyne incognita* was controlled by the application of the fungus *Paecilomyces lilacinus* (Thom) in soil infested with this nematode (Jatala et al. 1980). *Nematophthora gynophila* infests females and inhibits cyst formation. *Verticillium chlamydosporium* parasitizes immature eggs as well as chained L1 or L2 (Irving and Kerry 1986). The purpose of this study was: i) to isolate and identify potential biological antagonists of *H. avenae*cysts from different cereal regions of Algeria; ii) to determine the toxicity of *Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp. with respect to *H. avenae*eggs.

Methods

Nematode locations, sampling and extraction

Soil sampling of cereals naturally infested with *H. avenae*was carried out in four regions located in two different bioclimatic stages: Sidi Amar (Tipaza) and Mouzaia (Blida) in sub-humid; Khroub (Constantine) and Dahmouni (Tiaret) in semi-arid regions. These bioclimatic stages are distinguished by average annual temperatures and precipitation. In the sub-humid bioclimatic stage, the annual average temperature is 19°C and the annual average rainfall varies from 600 to 800 mm. In the semi-arid bioclimatic stage, the average annual temperature is 7°C in winter and 27°C in summer with an annual average of 17°C and an annual rainfall of 350mm. Cereal soil samples (durum wheat, soft wheat and barley) were collected between July and August 2018 from twelve plots: four in the region of Sidi Amar, three in the region of Dahmouni, three in the region of Khroub and two in the region of Mouzaia. Sampling was conducted at a depth between 0 and 30 cm using a auger (Rivoal 1986).

About ten soil samples were taken from each plot; each sample consists of five basic samples. All samples were carefully packed in plastic bags on which the coordinates of the sample were indicated (date and parcel number) and then stored at 4°C. In the laboratory, the soil was previously air-dried. The extraction of cysts was done with the Fenwick apparatus (1940).

Isolation and identification of fungi

The isolation of the fungi was carried out on about fifteen cysts disinfected in a 1% NaOCl solution for five minutes and rinsed three times successively with sterile distilled water (the first rinse at 2 minutes, the second at 3 minutes and the third at 5 minutes). The cysts were then dried for 10 minutes on sterile Wattman paper. They were then placed in 90 mm diameter Petri dishes on agar malt medium (20 g of malt extract, 10 g of agar and 1 liter of distilled water) (Rappily 1968), at the rate of one cyst per box. Fifteen repetitions were therefore carried out for each region. All these operations were carried out under a laminar flow hood in order to avoid external contamination. After 72 hours of incubation at 25°C, the cans were examined under binocular magnification every 24 hours for the first ten days, once a week later. After one month, the percentage of cyst infection by fungi was calculated. The fungi, which developed from the cysts, were isolated and purified on an agar medium. From these isolates, monosporal cultures were made on Malt medium and incubated at 25°C.

The identification of isolated fungi was done based on macroscopic and microscopic criteria. Macroscopic observations concerns the color and appearance of the culture. Microscopic observations (Leica BM type optics) were made on culture fragments containing the fructification forms, colored with lactophenol blue, placed between the blade and the slide and fixed by a rapid passage on the flame of the benzene beak. The observations concern the morphology of mycelium, conidia, conidiophores and chlamydospores. The genera of the individual fungi were identified using Barnett and Hunter(1972).

Preparation of fungal filtrates

Three strains of *Fusarium* spp. (M2, M9 and M12), two strains of *Penicillium* spp. (SA5 and SA9) and two strains of *Aspergillus* spp. (D3 and EK14) isolated from cysts belonging to the respective populations of Mouzaia (M), Sidi Amar (SA), Dahmouni (D) and El Khroub (EK), were assessed for their toxicity to *H. avenae*. A fragment of each fungal isolate was collected from the growing area and deposited in 250ml vials containing 100ml of YES liquid nutrient medium (composed of 40g sucrose, 20g yeast extract and 1000 ml distilled water). These vials were incubated for 14 days in the dark at a temperature of 26°C. After this time, the culture medium was filtered using Wattman paper of diameter 11 mm, which prevents the passage of all contaminants. The nematicidal effect of the evaluated isolates was tested with aqueous filtrate which is diluted in sterile distilled water at 25, 50 and 75%. Sterile distilled water was used as a control for comparison.

Analysis of fungal culture filtrates

The identification of toxic metabolites in the filtrate of fungal cultures was achieved through thin-layer chromatography (TLC) which was based on the differential migration of molecules according to their size, shape, mass, charge, solubility or their absorption properties. After 14 days of incubation, we got rid of the biomass formed by filtering the YES medium through a filter paper. Thus, 50ml of the filtrate obtained was added to 100, 50 and 30ml of chloroform respectively. The whole was agitated for 30 min. We then allow the mixture to settle down using a settling bulb in order to separate the aqueous phase from the chloroformic phase. The three chloroformic phases thus extracted were filtered on a filter paper and then concentrated with a steam rotovator until a volume of 2 to 3 ml was obtained. These extracts were kept away from light at 4°C until use them.

Toxicity of fungal filtrates on *H. avenae*eggs

*H. avenae*eggs were deposited in hemolysis tubes. Each tube received approximately 100 eggs to which we added a 100µl filter suspension to be tested of each strain of *Fusarium* sp. *Penicillium* spp. and *Aspergillus* spp. for all three concentrations (C1: 25%; C2: 50%; C3: 75%). Eggs were therefore subjected to four different treatments (control, *Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp.). Each treatment was repeated four times. Egg mortality were estimated after 6, 24 and 48 hours of incubation at 22°C, by periodic Meldola blue coloring at 1‰ which has the characteristic of crossing the cuticle of dead eggs and coloring their contents (Ogica and Estey 1974). Next, colored and uncolored eggs were counted under an optical microscope.

Statistical analysis

In order to study the correlation between the genera of the isolated fungi with the populations of *H. avenae* studied, we used a multi factorial analysis from statistical software (PAST vers.1.37) (Hammer et al. 2001).

Results

Fungi isolated. Forty-six fungi were isolated and identified primarily at the genus genus level. They belong to the class Zygomycetes (*Rhizopus* spp. and *Rhizomucor* spp.) and Deuteromycetes (*Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Ulocladium* spp.) (Figure 1). Based on morphological criteria, conidiophores of *Aspergillus* spp. ends in a bulge with sterigmas bearing single-celled and globoid conidia chains (Figure 1A). *Fusarium* spp. was characterized by slightly arched pluricellular macroconidia and ovoid to oblong unicellular microconidia *Penicillium* spp. was characterized by prolonged conidiophodes of phialids which carry at their apex long chains of unicellular conidia, globoid to ovoid (Figure 1C). For *Rhizomucor* spp., the hypha has no or few partitions and the zygospores were formed in the round, dark brown to black, aerial hyphae. *Rhizopus* spp. is characterized by non-septate hyphae, continuous-growing conidiophores that give rise to sporangia. The conidiophore of *Ulocladium*spp. was simple and branched and the conidia have a zig-zag appearance, of brown-black color, with a rough and cylindrical wall divided into several cells (Figure 1F).

Fungi frequency. *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. are the most commonly associated with *H. avenae*cysts collected in the four prospected regions. The highest diversity was observed in Dahmouni with the following genera *Aspergillus* spp., *Ulocladium* spp., *Rhizopus* spp. and *Rhizomucor* spp. which would be specific to this region. The highest percentage of infected cysts was found in Mouzaia (87%), whereas, the lowest in El Khroub (60%). Except the three genera *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp., all other fungi were isolated with a frequency of less than 30%. All these fungi were considered secondary parasites. Factor analysis of components (AFC) shows that axis 1 contributes 57.2% of information, while axis 2 contributes 41.4%. The AFC and Hierarchical Upward Classification (CAH) highlight three groups. The first group corresponds to the presence of the genera *Ulocladium* spp., *Rhizomucor* spp. and *Rhizopus* spp. with the region of Dahmouni. The second includes the genus *Fusarium* spp. with the region of Mouzaia, while the third includes *Aspergillus* spp. and *Penicillium* spp. with the regions of El Khroub and Sidi Amar.

Thin-layer chromatography. The results of thin-layer chromatography (TLC) were positive. Indeed, the reading of the two TLC plates of the different fungal isolates (Figure 2) showed a presence of dark blue and light blue fluorescence which means a migration of metabolites at the silicagel plate. In the absence of standards, it was impossible for us to determine the substances extracted from the migrating fungi.

Toxicity of fungal culture filtrates on *H. avenae*eggs. In our study, the percentage of mortality of eggs was 100% for all concentrations of fungal filtrates, 6h, 24h and 48h after inoculation. These results were confirmed clearly under a microscope when the eggs of *H. avenae* are colored in Meldola blue. Indeed, in the treatment with *Fusarium* spp. (M12) filtrate at the C1 concentration after 6h, the eggs were colored (dark), non-viable and undisturbed (Figure 3A). In *Fusarium* spp. (M2) filtrate treatment at the C2 concentration after 48h, we observe dead or non-viable colored eggs with one or more vacuoles (Figure 3B). With *Aspergillus* spp. (D3) filtrate at the C3 concentration after 24h, the eggs were colored (dark) with degraded embryos (Figure 3C). The application of *Penicillium* spp. (SA5) filtrate at the C2 concentration after 6h resulted in the mortality of embryo eggs which were colored and not degraded (Figure 3D). All these observations were different from those obtained on the controls (distilled water). The latter have no mortality, since the appearance of the eggs after coloring with Meldola blue has an uncolored embryonic content (Figure 3E).

Discussion

Forty-six fungi belonging to different genera were identified from *H. avenae* cysts populations from four cereal regions. These fungi belong to four orders. They are opportunists (Ownley and al. 1983; Dackman and Nordbring-Hertz 1985). Opportunistic fungi or secondary parasites are represented by Zygomycetes such as *Rhizopus* spp. and *Rhizomucor* spp. and several Deuteromycetes such as *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Ulocladium* spp..

The fungi isolated in our study were found all over the world in association with several cyst nematodes (*Heteroderidae*), suggesting that this microflora is specific to them (Willcox and Tribe 1974; Kerry and Crump 1977; Kerry 1980; Nigh et al. 1980; Ownley Gintis et al. 1983; Crump and Kerry 1987; Crump 1991; Crump and Flynn 1995; Chen et al. 1996). According to Kerry et al. (1982), opportunists such as *Penicillium* spp. infect only a small number of eggs and colonize only dead females. Note that some of the isolated fungi are associated with cereals.

All fungi isolated from the four Algerian regions surveyed have a frequency ranging from 13% to 66% and are considered secondary parasites (Dackman and Nordbring-Hertz 1985; Kerry 1988b). The association of *Fusarium* spp. with *H. avenae* suggests that this fungus likely has a role in the natural regulation of populations of this nematode. Its highest frequency in the Mouzaia region could result from bioclimatic conditions favorable to its development and especially from the monoculture of cereals (wheat or barley) practiced there.

However, some of them have been the subject of much work. They showed an inhibitory action against the mobility of infesting larvae (L2) gall or cyst nematodes. According to Kerry (1988a), more than 150 species of fungi have been identified on cysts, females and eggs of eight species of cyst nematodes in the soil, while about 20 species have been found on the eggs of *Meloidogyne* spp. (Chen et al. 1996). The 76% rate of cyst colonization obtained in our experiment corroborates the results revealed by Clovis and Nolan (1983) and Tribe (1980) who estimate that the cyst rate, eggs and females of cyst nematodes colonized by fungi in agricultural soils range from 10% to 90%.

The colonization of cysts, eggs or females by these fungi may affect their viability, which may explain the mortality of eggs in the cysts of the different populations studied. For example, Nigh et al. (1980) report that there are reports that indicate that nematode viability was severely reduced after being colonized by some opportunistic fungi.

At the soil level, populations of opportunistic fungi associated with nematodes may be significantly higher than populations of obligatory parasitic fungi (Yu 1989). The importance of regulating plant parasitic nematode populations in soil is positively related to the population of all fungal parasites, including opportunists (Kerry 1988b).

Under laboratory conditions, we demonstrated the toxicity of three strains of *Fusarium* spp., two strains of *Penicillium* spp. and two strains of *Aspergillus* spp. with regard to *H. avenae* which causes high nematode egg mortality. The mortality induced by the filtrates of these seven fungal strains is therefore a major potential in the control of this nematode. Indeed, the work of Ciancio (1995), Anke and Sterner (1997), Nitao et al. (2001) showed the toxicity of secondary metabolites produced by endophytic fungi, such as *Fusarium oxysporum*on *Meloidogyne incognita*, and toxins from various *Fusarium* spp.. that can reduce the viability of nematodes. Djian et al. (1991) have shown that it is monocarboxylic volatile fatty acid, which is the acetic acid that occurs abundantly during the growth of telluric fungi such as *Paecilomyces lilacinus* and *Trichoderma longibrachiatum* on a liquid medium and inhibits larval mobility infesting and hatching nematode eggs.

Fusarium spp., *Penicillium* spp. and *Aspergillus* spp. act either by the toxic action against the eggs of *H. avenae*, or by the inhibitory or destructive action of the eggs of nematode populations (Ameen 1991; Hallmann and Sikora 1996; Khan 1999; Sharma 1999; Wang et al. 1999; Costa et al. 2001; Randhawa et al. 2001. Similar results to ours were obtained by Molina and David (1986). Both authors showed that the purified extract of *Penicillium* spp., *Penicillium oxalicum* and *Penicillium anatolicum* Stolk has nematicidal activity at 100 and 200ppm. The efficacy of the fungi could therefore result from a toxic effect that confirms the results of several studies that have shown the interest of these antagonistic against plant pest nematodes.

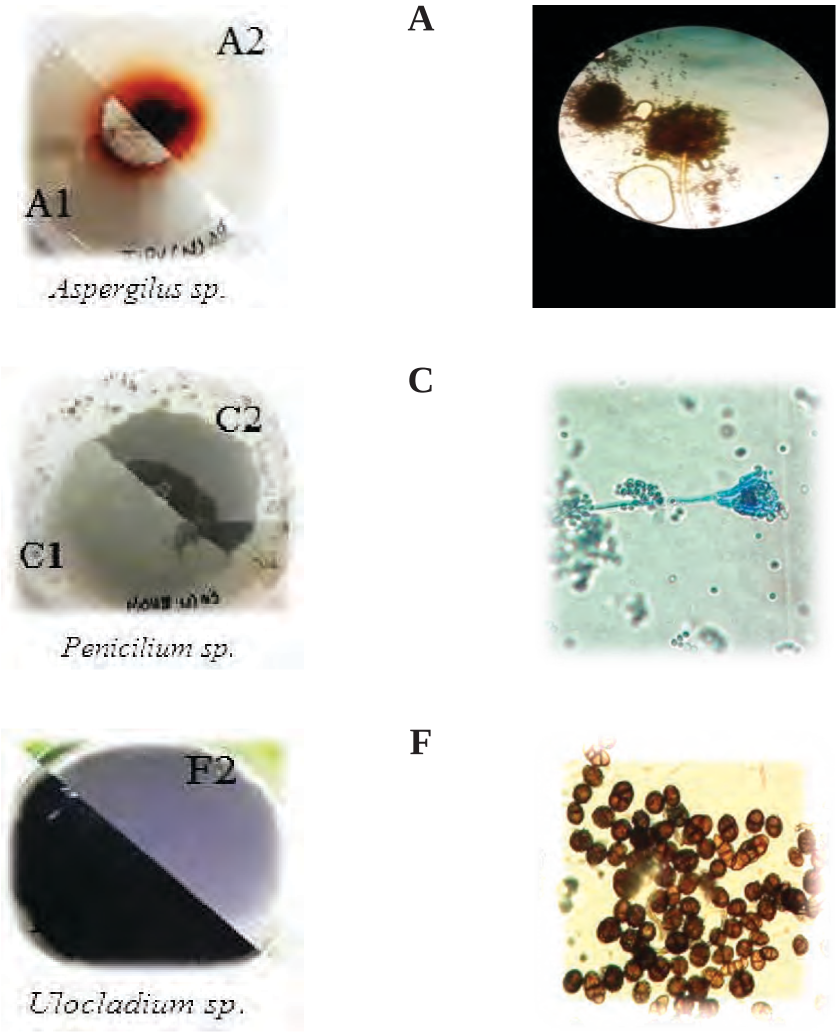

Figure 1. Macroscopic and microscopic observations of different genera isolated from the cysts of *H. avenae.* Top of the Petri dish: A1, C1, F1; Bottom of the Petri dish: A2, C2, F2; A: *Aspergillus* spp.; C: *Penicilliums* pp.; F: *Ulocladium* spp.

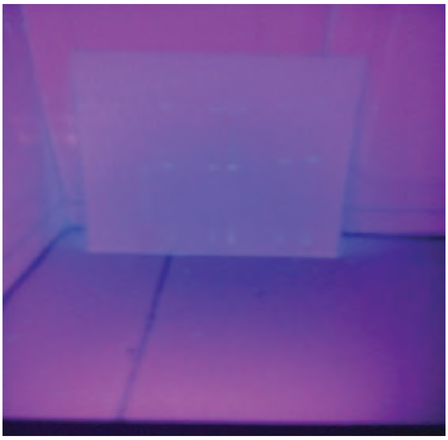

Figure 2. Migration of three chloroformic fungal extracts of *Fusarium* spp. (M.: Mouzaia).

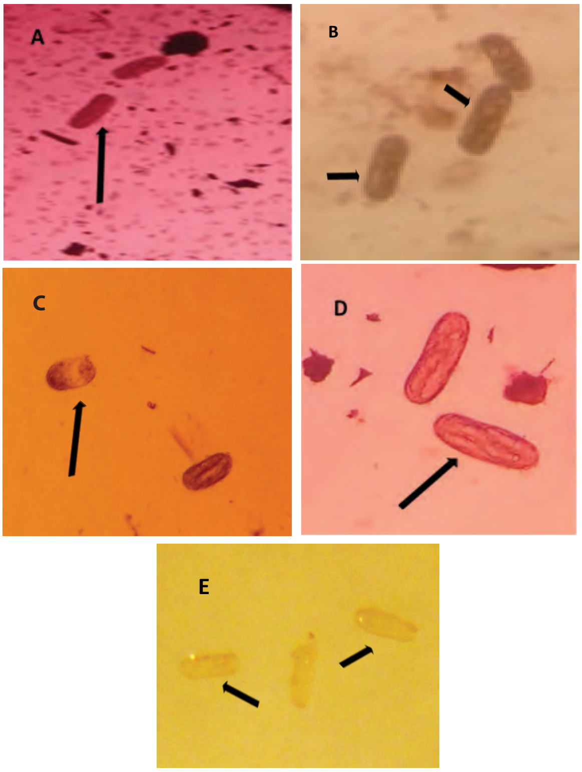

Figure 3. Appearance of eggs and chained larvae of *Heterodera avenae* after coloring Meldola blue at 1‰.
A: embryo eggs, colored (dark), non-viable and not degraded; **B:** dead eggs or viable eggs, colored and exhibiting one or more vacuoles; **C:** colored egg (dark) with a degraded embryo;
D, colored and undiluted embryos; **E:** colorless (clear) viable embryo (control). Eggs are indicated by an arrow.

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Preliminary evaluation of the impact of nematode antagonistic and endophytic fungi against the beet cyst nematode *Heterodera schachtii* in the greenhouse

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Summary

Plant parasitic nematodes (PPNs) have a great impact on the agriculture sector by causing significant losses every year. With different management strategies being applied to control PPNs, biocontrol methods have shown the ability to be used as an efficient management tool i.e., antagonistic fungi against PPN populations. Several newly discovered endophytic fungi have been reported to infect the females and eggs of endo-parasitic nematodes. This study was conducted to examine the bi-functional lifestyle of *Ijuhya vitellina*, *Monocillium gamsii* and two Pleosporalean strains JKI72994 & JKI72728 against the beet cyst nematode *Heterodera schachtii* and their growth promoting effect on sugar beet under greenhouse conditions. The preliminary results showed that plants inoculated with the Pleosporalean strain JKI72994 and *M. gamsii* had an increase in total biomass and their root biomass. Two of the four fungi (*Ijuhya vitellina*, *Monocillium gamsii*) showed levels of indole-3-acetic acid (IAA) production.

Introduction

Plant parasitic nematodes (PPNs) have great economic impact on the agriculture sector. The economic losses caused by PPNs is estimated between 80-120 USD Billion per year (McCarter 2009), different management strategies are applied to control PPNs. As the awareness of the hazards of pesticide use increases internationally, it has resulted in the improvement of non-chemical based management strategies. Among others, biocontrol methods of PPNs have shown the ability to be used as a management tool and should be studied more extensively and in more detail (Kohl et al. 2019). An example of biocontrol agents is the use of antagonistic fungi against PPN populations (Poveda et al. 2020). New fungal endophytes are continuously being discovered and various fungi have been reported as natural enemies of PPNs. A group of these fungi infect females and egg contents of endo-parasitic nematodes i.e., cyst and root-knot nematodes (Stirling 2014).

Several studies show that endophytic fungi have an influence on plant development and contribute to plant survival (Schouten 2016). Fungi can have an impact on plant population through increasing general plant health. That is by increasing plants tolerance to abiotic and biotic stresses and increasing plants biomass (Rodriguez et al. 2009; Schouten 2016). For example, it was shown that numerous plant-associated fungi produce phytohormones i.e., auxins, cytokinins and gibberellins. These phytohormones are involved in plant growth promotion and aid the plant to tolerate abiotic and biotic stresses (Aloni et al. 2006; Rodriguez et al. 2009; Mei and Flinn, 2010). Accordingly, fungi can be explored as a tool for biocontrol and to promote plant growth in the presence of abiotic and biotic stresses such as drought, heat, or plant pathogens like cyst nematodes.

The described species *Ijuhya vitellina, Monocillium gamsii* (Ashrafi 2017a, b) and two Pleosporalean strains JKI72994 & JKI72728 were initially isolated from naturally infected cyst nematodes and examined *in vitro*, where Koch's postulates were proven, and showed an antagonizing nature against cyst nematodes. Additionally, the Pleosporalean strains JKI72994 & JKI72728 have been reported in independent ecological studies as root endophytic fungi associated with different plant species including wheat and representatives of Brassicaceae (Glynou et al. 2016). These studies led us to propose that these recently discovered fungi may have a bi-functional lifestyle. They parasitize cyst nematode eggs but are also found as plant symbionts (endophytes).

Accordingly, the current study was conducted to: (1) examine the antagonizing effect of these fungal strains against the beet cyst nematode (BCN) *Heterodera schachtii*, (2) evaluate their impact on growth promotion of sugar beet under greenhouse conditions.

Methods

Pot experiments were established using a sterilized soil substrate. Plants were inoculated with fungal isolates, and infective second stage juveniles (J2s) of *H. schachtii* with the following treatments: (1) fungus and nematode (2) fungus (3) nematode alone (control), and (4) only plant (no inoculation). Plants were grown in a greenhouse (20°C photoperiod of 16 hours). Each treatment consisted of 10 replicates and the experiment was repeated twice. The experiment was kept for 4 months, then the plants were uprooted, and cysts were extracted from each individual pot. Growth parameters were recorded i.e., plant height, shoot height, root length plant dry/fresh weight and biomass.

In addition, the detection of auxin production level of the fungal strains was tested following the Salkowski colorimetric assay (Gordon and Weber 1951) to determine whether they produce indole-3-acetic acid (IAA) according to Naureen et al. (2022).

Results and discussion

The preliminary results showed that the total biomass was significantly the highest in plants inoculated with the fungal isolate JKI72994, followed by plants inoculated with the same fungus and nematode, and plants inoculated with *M. gamsii*. The root biomass was significantly highest in plants inoculated with the fungal isolate JKI72994 followed by plants inoculated with *M. gamsii*. The antagonizing effect of the fungal strains on BCN and their control efficacy is currently under evaluation.

Initial results showed the detection of IAA in 2 of the fungi studied here, namely *I. vitellina* and *M. gamsii*. To confirm our finding of IAA production by these fungi, further experiments and tests will be conducted. The preliminary findings open the potential to test these fungal isolates for other phytohormones that may be produced.

With the current available data, we can presume that some of the studied fungal isolates improve plants growth and aid the plant to tolerate the BCN stress. Generally, the plant-fungi-nematode, plant-fungi and fungi-nematode interactions is an aspiring field of research to be studied in-depth.

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Management of *Meloidogyne enterolobii* in a maize cropping system using legume based rotation

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Introduction

The constant increase of the human population necessitates that more food must be produced to ensure food security worldwide, while cereal crops are an important source of human and animal food (FAO 2017). Cereal production, reduced by 4.6% in 2019 in comparison to 2018 (FAO 2020) in Africa placing risk to food security in this continent. Production of maize (*Zea mays*) as the main grain crop in South Africa is hampered by a variety of diseases and pests including plant parasitic nematodes (PPNs). This group of nematodes are a key limiting biotic factor in maize cropping systems (Fourie et al. 2017; Mc Donald et al. 2017). Previous research found that root-knot nematodes caused up to 60% losses in South African maize crops (Riekert and Henshaw 1998). Among other eco- friendly control strategies, crop rotation is one of the methods that could reduce PPN populations in maize-based agriculture systems. However, most crops, particularly legumes that are used in rotation with maize are subjected to the same nematode pest as maize (Fourie et al. 2017; Dababat and Fourie 2018). Therefore, it is critical to identify rotational crops in maize-based cropping systems while suppressing PPN levels. Sainfoin (*Onobrychis viciifolia*) could be a suitable alternative, due to its chemical features *i.e.* the formation of condensed tannins, which was shown to have anthelmintic effect against ruminant gastrointestinal parasitic nematodes (Novobilský et al. 2013; Mueller-Harvey et al. 2019).

Material and Methods

This study was designed to evaluate the rotation effects of different legumes with maize on a *Meloidogyne enterolobii* population, compared to monoculture maize. Different legume cultivars were used to assess their impact on nematode population densities: cultivars of sainfoin including Esparsette, Perly, Taja and Visnovsky), the soybean cultivar, and the alfalfa cultivar BAR7. The maize cultivar P-2432-R was used in all experiments. The tomato cultivar Moneymaker was used as a positive control for nematode infection. Pots (0.5-L capacity) were inoculated with 500 eggs and J2 from an *in vivo* reared *M. enterolobii* population. All plants were uprooted five weeks following nematode inoculation. The aerial part and roots of each plant were incorporated into the soil of the corresponding pot (4–8 cm deep) by cutting the plant material into small (2 cm) chunks. One week later, pots were seeded with one maize seed. The tomato pots were re-sown with tomato. The experiments were terminated in five weeks by extracting nematodes from roots. Different criteria such as nematode population density, reproduction factor value of the nematode, and different plant growth parameters were determined. The experiments were repeated twice.

Results and discussion

The data collected showed that rotation of sainfoin (Esparsette)/maize had the lowest number of *M. enterolobii* eggs and J2 per root system in both experiments. Among the treatments (excluding tomato), soil amendment and rotation of sainfoin Taja/maize produced the most eggs and J2 in the first trial, whereas monoculture maize produced the most eggs and J2 in the repeat trial. In the first trial, two sainfoin cultivars (Perly and Esparsette) and alfalfa had significantly lower numbers of eggs and J2 than monoculture maize. However, in this experiment, soil amendment and rotation of sainfoin (Taja) with maize significantly enhanced the root-knot nematode population. In the first trial, no significant changes ($P \leq 0.05$) were identified when soybean was rotated with maize vs monoculture maize. In the repeat trial all treatments showed significantly lower number of root-knot nematodes compared to the monoculture maize. When sainfoin Perly, alfalfa, and sainfoin Esparsette utilized as rotation with maize, nematode population densities reduced by 7, 54, and 81%, respectively. Similar to the first trial, soil amendment and rotation of sainfoin (Esparsette)/maize reduced nematode population density by 60%, followed by alfalfa/maize (43%). This finding emphasizes the beneficial function that sainfoin may have in grain-based sequences to reduce *M. enterolobii* damage.

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Session IV

Basic research trends in cereal nematodes (such as pathogen diagnostics, phylogenetic studies, and plant nematode interactions)

Study of wheat fields in the south-west province, Iran, unfolds the spread of cereal cyst nematode in this region

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Summary

Among the identified species of cyst-forming nematodes belonging to the *Heterodera avenae* group, *H. filipjevi* is the most prevalent species in cereal fields in Iran. The wheat fields of Kohgiluyeh and Boyer-Ahmad province in the south west of Iran were surveyed, 150 composite soil and root samples were randomly taken during 2017-2020 along with recording the geographical coordinates of the sampled locations using a GPS. The samples were subjected to nematology methods, the species was identified based on morphological and morphometric features and molecular characters. The results revealed presence of cereal cyst nematodes, *H. filipjevi* in 56% of samples, however the occurrence of CCN were almost closed in rain-fed and irrigated fields by 55 and 57.6% respectively. The average population density of eggs and second stage juveniles of *H. filipjevi* were 2.6 and 2.51 /g soil, in rain-fed and irrigated fields respectively. Whilst the highest and lowest mean populations was 4.9 and 1.2 in Basht and Gachsaran in the warm areas respectively. *Heterodera filipjevi* was the only identified cereal cyst nematode species in this study which its distribution in the region is discussed.

Introduction

Kohgiluyeh and Boyer-Ahmad Province is located in southwestern of Iran. The total area under irrigated and rain-fed wheat cultivation in this provinces, is 99000 hectares that about three of quarters of the areas are under rain-fed cultivation. Meanwhile the annual wheat production is estimated 136,680 Tons, about 3,122 and 912 kg / ha in irrigated and rain-fed respectively in 2020 (Ahmadi et al. 2021).

Among the cereal cyst nematodes, *Heterodera filipjevi* is the most common species of cereal cyst nematodes (CCNs) in most cereal growing areas of Iran. Five species belonging to the *Heterodera avenae* group consisting of *H. avenae*, *H. filipjevi*, *H. hordecalis*, *H. latipons* and *Heterodera* sp. were identified and reported from cereal fields and grasslands in Iran (Tanha Maafi et al. 2007).

So far, no comprehensive study has been conducted on the identification and distribution of cereal cyst nematodes in Kohgiluyeh and Boyer-Ahmad Province, especially in tropical regions of the province, although *H. filipjevi* was reported from the colder regions of this province (Abdollahi 2008). However in the extensive studies conducted in wheat fields of Isfahan and Chaharmahal and Bakhtiari provinces the neighbors of Kohgiluyeh and Boyer-Ahmad province were shown severe soil infestation and CCN distribution in both provinces. (Karimipour Fard *et al*. 2016, 2017a).

Trials on assessment of yield loss due to *H. filipjevi* indicated significant reduction of grain yield by 24.8%, 24.8% and 20.4% in Back-cross Rowshan, Pishtaz and Parsi cultivars, respectively under field conditions (Karimipour Fard et al. 2017b). We studied identification, the spreading of CCN based on the geographic information system (GIS), population density in both irrigated and rainfed wheat fields in cold and tropical regions of the province.

Methods

A total of 150 composite soil and root samples were randomly taken from wheat fields of different counties of Kohgiluyeh and Boyer-Ahmad province during 2017-2020 along with recording the geographical coordinates of the sampled locations using a GPS. The sampling was done during the grain filling period until harvesting time. The soil samples were processed by the Fenwick can for extraction the cyst-forming nematodes.

Species was identified based on morphological and morphometric features and molecular characters. To identify the specimens, permanent slides were made by excising the vulval cone region of cysts and mounting them in glycerin jelly, and by fixing J2 from each cyst in TAF and mounting them in glycerin. The ITS-rDNA gene was amplified with the forward TW 81and reverse AB28 primers. Specific primers for identification of *H. filipjevi* (FILI-COIF and FILI-COIR) were also used (Toumi et al. 2013). The population densities of CCNs and the mean of population densities of samples belonging to each districts were evaluated as well.

Results

The results revealed *Heterodera filipjevi* was the only species of cereal cyst nematodes identified in Kohgiluyeh and Boyer-Ahmad province. Amplification of *H. filipjevi* ITS-rDNA regions produced a fragment of 1000 bp. The results of comparing the obtained sequences with the sequences in the World Gene Bank showed that the sequence of these regions of cereal cystic nematode ribosomal RNA was 99% similar to the sequences deposited in NCBI. Using *H. filipjevi* specific primers, amplified a fragment of about 250 bp (data not shown), which was consistent with previous results of identifying *H. filipjevi* using a specific primer (Toumi et al. 2013).

Among the 75 soil samples, including 49 soil samples from the rain-fed and 26 samples from the irrigated wheat fields of Kohgiluyeh and Boyer-Ahmad province, 42 soil samples (56%) contained cereal cyst nematode, *H. filipjevi*. The percentage of infested rain-fed and irrigated wheat fields were 55 and 57.6, respectively. The average population of eggs and second stage juveniles (J2s) in cysts was estimated as 2.6 eggs and J2s /g of soil. The minimum population density of *H. filipjevi* (0.5 eggs and J2s/g of soil) was found in wheat fields of Tal Khosrow region in Boyer-Ahmad county, Bid-Zard region of Gachsaran county, Lirab region of Basht county and Bijgan and karyak regions of Dena county. The rain-fed wheat field (Dehdasht cultivar) of Lirab region in Basht county and the rain-fed wheat field (Azar 2 cultivar) from Chenarestan-Sofla region of Boyer-Ahmad county, with 13.7 and 12.9 eggs and J2s/g of soil, had the highest mean population density of *H. filipjevi*, respectively. The average population of eggs and J2s in the both rain-fed and irrigated wheat fields infested with the *H. filipjevi* in all counties of the province were estimated to be 2.6 and 2.51 per gram/ soil, respectively. In total rain-fed and irrigated wheat fields by counties, the highest and lowest mean populations of eggs and J2s of *H. filipjevi* was 4.9 and 1.18 belonged to Basht and Gachsaran counties, respectively (Table 1).

Table 1. Incidence of *Heterodera filipjevi* in irrigated and rainfed wheat fields of Kohgiluyeh and Boyer-Ahmad Province in soil and root samples collected during 2017-2020.

District	No. of total soil samples		No. of infested soil samples		Mean and range of eggs and J2/g soil		Mean and range of eggs and J2/g soil (sum of irrigated and rainfed soil samples)
	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	
Boyer-Ahmad	6	29	1	15	2 (2)	2.7±2.97 (0.5-12.9)	2.35±2.8 (0.5-12.9)
Gachsaran	2	5	2	1	1.85±0.35 (1.6-2.1)	0.5 (0.5)	1.18±0.81 (0.5-2.1)
Basht	4	6	1	5	4.2 (4.2)	5.57±5.2 (0.5-13.7)	4.9±4.69 (0.5-13.7)
Dena	3	4	2	3	2.1±0.42 (1.8-2.4)	1.3±0.63 (0.5-1.6)	1.7±0.83 (0.5-2.4)
Choram	1	2	0	2	0	2.75±0.21 (2.6-2.9)	2.75±0.21 (2.6-2.9)
Kohgiluyeh	4	0	3	0	2.77±0.57 (2.1-3.1)	0	2.77±0.57 (2.1-3.1)
Lendeh	5	1	5	0	2.8±2.26 (0.6-6.58)	0	2.8±2.26 (0.6-6.58)
Bahmaei	1	2	1	1	1.8 (1.8)	2.6 (2.6)	2.2±0.56 (1.8-2.6)
Total	26	49	15	27	2.51±1.38 (0.6-6.58)	2.6±3.32 (0.5-13.7)	2.6±2.77 (0.5-13.7)

Discussion

Identification of *H. filipjevi,* corresponds to the previous report of this species from the cold part of the province (Abdollahi 2008). The results of studies on different initial population density of *H. filipjevi* on wheat (Sardari cultivar) in microplot trials showed that grain yield reduction occurred even at the lowest population density (2.5 eggs and J2/g soil) and reached a maximum grain yield reduction of 48% with an initial population density of 20 eggs and J2/g of soil (Hajihasani et al. 2010). Therefore, the results obtained from this study indicate that a decrease in grain yield is expected in wheat fields of Kohgiluyeh and Boyer-Ahmad province. Also, due to the fact that the existing reports show increased damage of cereal cyst nematodes, including *H. filipjevi* in rainfed cultivation and under drought stresses conditions in most wheat producing countries (Braun et al. 2009, Karimipour Fard et al. 2015) and also considering the fact that most of the area under wheat cultivation in Kohgiluyeh and Boyer-Ahmad provinces are rainfed, the importance of damaging *H. filipjevi* in wheat fields of the province becomes more apparent. Conduction of additional research to evaluate the amount of damage and yield reduction caused by *H. filipjevi*, especially in the rainfed cultivars of the province is recommended.

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Interaction of root lesion nematode (*Pratylenchus thornei*) and crown rot fungus (*Fusarium culmorum*) associated with spring wheat resistance under simulated field conditions

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Summary

The root-lesion nematode, *Pratylenchus thornei*, and the crown rot fungus, *Fusarium culmorum*, cause tremendous damage to a wide range of grain crops, resulting in significant economic losses in the global yield production. The presence of both pathogens simultaneously in cereal fields puts more pressure on breeding programs, restricting new resistant cultivars to express their potential. This study investigated 150 spring wheat lines of the 18KASIB-CORE nursery for their host suitability to *P. thornei* and *F. culmorum*, inoculated singly or in combination. Forty-eight lines were resistant to *P. thornei*, while 16 lines were moderately resistant to *F. culmorum*. Among these, nine lines maintained their resistance reaction against the complex disease involving both pathogens, while only four lines provided resistance against individual and combined infestations. Infestation scenarios of both pathogens were monitored for each resistant variety and predicted using both reproduction factor and disease index. *Pratylenchus thornei* downgraded all resistant lines to *F. culmorum*, facilitating infestation and making their severity more pronounced (169% cumulative damage) with a negative impact on yield performance. These findings are useful for improving nematode × fungus resistance in wheat and selecting promising germplasm for breeding purposes against both diseases.

Introduction

Among cereal crops, wheat (*Triticum aestivum* L. and *T. durum* Desf.) occupy a prominent position regarding production, nutrition source, and acreage patterns, especially in developing countries (Nicol et al. 2011). Several soil-borne pathogens can negatively impact wheat's agronomic yield parameters, including the root-lesion nematodes (RLN, *Pratylenchus* spp.) and the crown rot fungus (CRF, *Fusarium* spp.).

The genus *Pratylenchus* is globally prevalent, with many species associated with wheat (Castillo and Vovlas 2007). They are obligate parasites that can attack different crops, causing significant damage in the order of 190 million US $ per annum in Southern and Western Australia (Vanstone et al. 2008). *Fusarium culmorum*, known as crown rot fungus (CRF) is one of the soil-borne pathogens that are widely distributed and cause tremendous damage to a wide range of host plants (Nelson et al. 1993; El-Kazzaz et al. 2008). This damage negatively impacts wheat production areas valued at billions of dollars in yield losses (Windels 2000).

Resistant and tolerant wheat cultivars are the most eco-environmental and promising methods to manage the RLN occurrence (Castillo et al. 1998; Mokrini et al. 2019; Seid et al. 2021). Many studies have reported several sources of resistance regarding wheat germplasm (Thompson et al. 2008; Toktay et al. 2012; Dababat et al. 2019), and they can be mainly obtained through a screening program of wild wheat relative accessions.

The interaction between root-lesion nematodes and *Fusarium* species has been reported previously (Castillo et al. 1998; Hajihassani et al. 2013) and it concerns different *Pratylenchus* species. Nematodes tend to increase the severity of diseases induced by the fungus, causing highly significant economic damage and yield losses (Powell 1971).

The objectives of this study were to (i) investigate the resistance of wheat lines against *P. thornei* and *F. culmorum* alone and in combination, (ii) estimate the effect of co-inoculations of root-lesion nematodes (mainly *P. thornei*) and *F. culmorum* under simulated field conditions on growth and yield of spring wheat, and (iii) predict the damage caused by the disease complex when either *P. thornei* or *F. culmorum* attacks first.

Methods

Plant Material: The 150 spring wheat lines were provided by the KASIB network through CIMMYT, Türkiye. Three independent trials were performed to investigate the host suitability of germplasms to *P. thornei*, *F. culmorum,* and co-inoculation complex. Each trial was repeated twice for data validation. Standard known check lines (CLs) were adopted for both the nematode and the fungus.

Inoculum preparation: *Pratylenchus thornei* and *Fusarium culmorum* were extracted from infested wheat fields in Bolu province, Türkiye. The nematode suspension was used to inoculate wheat seedlings 1 week after planting, at a density of 200 individuals per ml of water (Toktay et al. 2012). 1 ml conidial suspension of *F. culmorum* at a rate of $1{\times}10^6$ spore per ml of water was used for inoculation. (3 cm above soil level) (Erginbas-Orakci et al. 2016). Regarding the interaction trial (*P. thornei* × *F. culmorum*), both inocula were applied simultaneously at the same individual rates and kept conditioned as previously mentioned.

Plant growth and disease parameters: The reproduction factor (RF) and the disease index (DI) were calculated for each trial and classified according to a 1-5 resistance scale (R, MR, MS, S, and HS) (Dababat et al. 2016; Erginbas-Orakci et al. 2016, 2018). In addition, several plant growth variables were measured, including plant height (cm), shoot dry weight (g), root dry weight (g), root length (cm), and grain yield (g tube^{-1}).

Statistical analyses: Data were processed using analysis of variance (ANOVA) after being normalized by the Anderson–Darling normality test (Stephens 1974). Population structure was distinguished by the Linear Discriminant Analysis (LDA) to determine putative groups of wheat lines based on their resistance, and susceptibility to *P. thornei* and *F. culmorum*. Polynomial regression analysis was established to reveal the correlation between *P. thornei* × *F. culmorum* interaction parameters (RF and DI) and the grain yield of the studied wheat line set.

Results and discussion

Spring wheat resistance against *Pratylenchus thornei* and *Fusarium culmorum*: Population structure based on Linear Discriminant Analysis (LDA) displayed five groups, depicting the resistance reaction to both *P. thornei* and *F. culmorum* among the 150 lines investigated (excluding CLs). Regarding the nematode, we found 48 resistant (R), 48 moderately resistant (MR), 24 moderately susceptible (MS), 16 susceptible (S), and 14 highly susceptible lines (HS) (Figure 1a). As for the fungus, we found 16 MR, 49 MS, 48 S, and 37 HS lines (Figure 1b). Dababat et al. (2019) evaluated 484 CIMMYT's spring wheat varieties for resistance to *P. thornei* of which 56 lines were classified as resistant under controlled growth chamber conditions, while Hekimhan (2010) stated that only 20% of wheat cultivars were able to resist *F. culmorum* constraints. The plant growth parameters were significantly reduced compared to the negative control due to the pathogens.

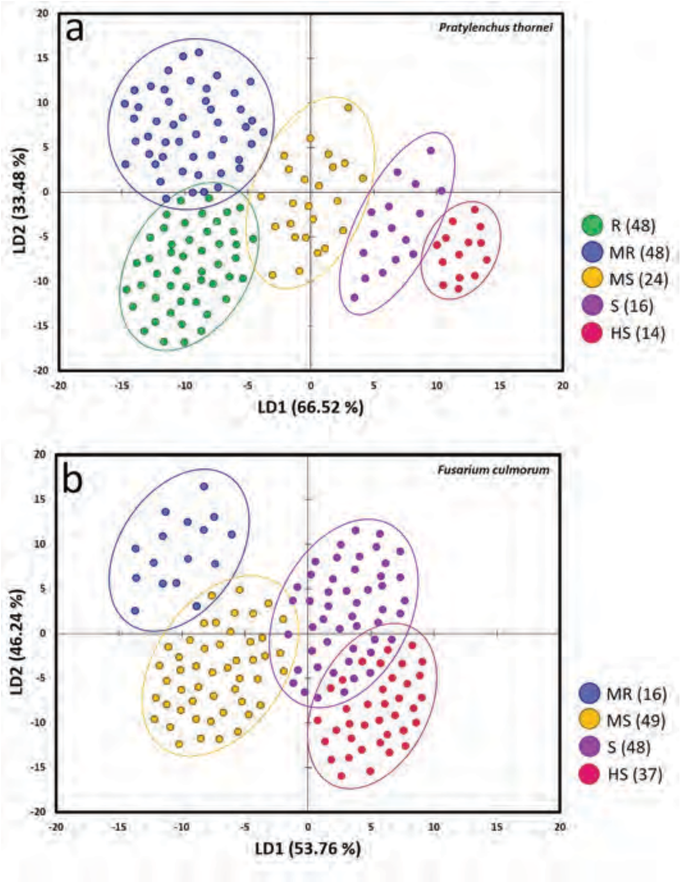

Figure 1. Linear Discriminant Analysis (LDA) showing the population structure for a set of 150 lines from the spring wheat nursery (18KASIB-CORE) based on the reproduction factor values; (**a**) LDA displaying resistance reaction (RR) ranking for *Pratylenchus thornei*. (**b**) LDA displaying resistance reaction (RR) ranking for *Fusarium culmorum*. LDAs were plotted by using the *pyLDAvis.sklearn* and *matplotlib.pyplot* modules of Python, integrated into PyCharm API. Abbreviations stand for: R = Resistant, S = Susceptible, HS = Highly Susceptible, MR = Moderately Resistant, MS = Moderately Susceptible.

Spring wheat resistance against RLN-CRF complex: Significant RF and DI increases were recorded due to the co-inoculation *P. thornei* × *F. culmorum*. Interestingly, the prior infestation by RLNs followed by CRF was predicted via resistance attributes to reach 81% (45% *P. thornei* + 36% *F. culmorum*) (Figure 2a), while *Fusarium culmorum* enhanced the virulence of *P. thornei* to cause up to 100% resistance reduction. Additionally, the fungus also obtained support for enough space to invade plants' roots by increasing its severity by 69%. Hence, the cumulative damage was predicted to be 169% (Figure 2b). The regression analysis has shown that there is a negative relationship between the pathogen indices (RF and DI) and the grain yield. Only four lines (Pavlodarskaya 11, Fiton C 50 ЧС, Lutescens 1519, and Lutescens1082) were able to maintain their resistance to both pathogens alongside their interaction, making them excellent sources for breeding purposes.

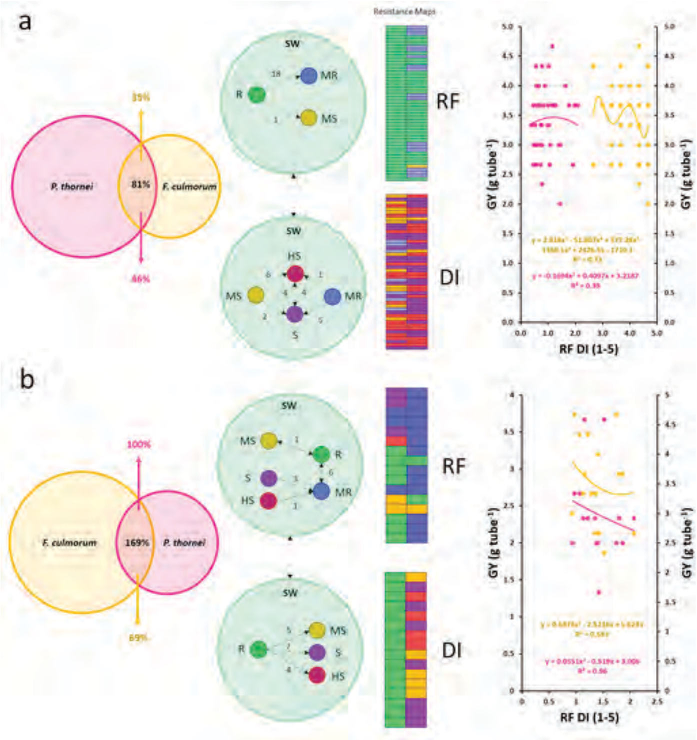

Figure 2. Contribution patterns and scenarios of *Pratylenchus thornei* × *Fusarium culmorum* on spring wheat resistance and yielding attributes. (**a**) Venn diagram depicting the damage (in percentage) caused when *P. thornei* infests first alongside resistance state changes which occurred between the associated 48 resistant SW plants; (**b**) Venn plot depicting the damage (in percentage) caused when *F. culmorum* infests first alongside resistance state changes occurred between the associated 16 SW plants. The numbers presented in SW green circles represents the converted lines from each resistance state to the other, caused by the pathogens damage according to the resistance maps. Polynomial regressions of grain yield (GY), *P. thornei* reproduction factor (RF), and *F. culmorum* disease index (DI) in SW nursery. R^2 values were considered significant at $P < 0.001$. The link represents the predicted polynomial regression model (rose for *P. thornei* and yellow for *F. culmorum*). Grain yield and equations were described in g tube^{-1}. Venn diagrams were constructed using the *matplotlib_venn* module in Python. Abbreviations stand for: R = Resistant, S = Susceptible, HS = Highly Susceptible, MR = Moderately Resistant, MS = Moderately Susceptible.

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Session V

Cereal nematodes genome and parasitism genes

Early molecular resistance response to simultaneous biotic and abiotic stresses in wheat

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Summary

Plants naturally face various interactions of many biotic and abiotic stresses that limit their growth and yield potential. Cereal cyst nematode *Heterodera filipjevi* and *Fusarium* spp. causing dryland root rot are important soil-borne pathogens (SBPs), causing significant yield losses to wheat in dryland regions. Plants have evolved some defense mechanisms to protect themselves from stressful conditions. Early response gene expression occurs transiently and rapidly in response to a wide variety of environmental stresses and activates plant defense pathways to produce pathogenesis-related (PR) proteins especially PR1 that is anti-oomycete and antifungal. Another early response gene is *ERD15* (Early Responsive to Dehydration15) that is rapidly induced in response to various abiotic and biotic stress stimuli. Regarding the importance of studying the interaction effect of biotic and abiotic stresses on the expression pattern of crucial genes involved in the early response of bread wheat to drought, *Fusarium culmorum* and *Heterodera filipjevi,* the expression pattern of *PR1* and *ERD15* genes was studied across 3 time points including 24 h, 48 h and 1 week after inoculation on two hexaploid bread wheat, variety (2-49) (Resistant to *F. culmorum*) and variety Silverstar (Resistant to *H. filipjevi*). The results revealed that both *PR1* and *ERD15* genes were up-regulated in response to different treatments in both varieties. Expression of *PR1* and *ERD15* was significantly higher under combination of treatments than gene expression under single stress conditions. Up-regulation of *PR1* and *ERD15* genes in variety 2-49 (resistant to *F. culmorum*) was higher under interactions of fungus with nematode and drought. Expression of *PR1* and *ERD15* genes in variety Silverstar (resistant variety to *H. filipjevi*) was higher under interaction of nematode stress with other stresses. Interestingly in the present study, a combination of drought and each pathogens attack caused a higher up-regulation in *ERD15* compared with up-regulation by each single pathogen stress. *ERD15* enhances SA-dependent defense by antagonistic crosstalk with ABA signaling and therefore improves *PR* genes induction. In the present study, *PR1* gene showed a higher up-regulation under the interaction of drought/pathogen stresses.

Introduction

Wheat production is facing with different biotic and abiotic stresses around the world. Drought as a shortage of water is a major abiotic stress causing losses in wheat growth and yield. *Heterodera filipjevi* is one of the most economically important CCN species reported to infect crops across different regions of the world. *Fusarium culmorum* is another soil borne disease causing root rot and crown rot in cereals particularly in wheat and barley (Ahmadi et al. 2022).

Under such situations, plants have to set a series of adaptation mechanisms ranging from cellular metabolism to physiological and developmental responses (Harbaoui 2018). Plant physiology, morphology and biochemistry are genetically controlled by a large set of genes. Expression of these genes is influenced by a number of factors, internal as well as external, including stage of plant development, diseases, drought, salinity, cold, and many others (Ziaf et al. 2016). In this way plant hormones such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA) play central signaling roles and regulate defense responses in plants (Jiang et al. 2010).

ERDs genes have emerged as a new class or group of genes, identified first time by Kiyosue et al. (1994). *ERD15* is a transcription factor for the up-regulation of the salicylic acid-dependent defense pathway and acts as an important negative regulator of abscisic acid. The negative effect of *ERD15* on ABA signaling enhances SA-dependent defense seen as improved induction of *PR* genes and leading to enhanced resistance to the pathogens (Kariola et al. 2006).

PR proteins production immediately after pathogen attack is one of the early defensive responses in plants. *PR* genes are also responsive to abiotic environmental stimuli, such as drought, salt, osmotic stress, low temperature and light conditions. It can also be understood that a defense gene can interfere in several resistance paths and cause resistance to many stresses.

Methods

Plant materials, growth and stress conditions: Two hexaploid bread wheat (*T. aestivum* L.) accessions including accession (2-49) (Resistant to *F. culmorum*) and accession Silverstar (Resistant to *H. filipjevi*) were used for gene expression analysis. Seeds were surface sterilized and germinated. Seven-day-old plants were exposed to single, dual and triple combinations treatments of drought, nematode and fungus stresses. Drought stress applied in present study included 40% of field capacity (Ahmadi et al. 2022). Cereal cyst nematode, *H. filipjevi,* sample collected by CIMMYT-Türkiye (Dababat et al. 2014) was used in this study, and each seedling was inoculated with 400 freshly hatched J2 of *H. filipjevi* (Ahmadi et al 2022). An identified isolate of *F. culmorum,* causing crown rot, from CIMMYT-SBP collection was used as the third stress factor (Ahmadi et al 2022). Each seedling was inoculated with *F. culmorum* spores suspended in water (1×10^6 spores mL^{-1}) (Erginbas-Orakci et al. 2016).

Experimental design and sampling: The experiment was conducted and analysed as a 2×2×2×2×3 factorial with 6 replicates (two biological and three technical) in a completely randomized design (CRD). Factors include accession (2–49 and Silverstar), two drought levels (40% stress and non-stress), two CCN levels (inoculated and non-inoculated), two crown rot levels (inoculated and non-inoculated), and sampling time (24h, 48h, and one week after treatment). Mean comparison was performed using the least significant difference test (LSD) ($P \leq 0.05$). Each biological replicate sample contained five seedlings. Sampling of wheat root tissue, for RNA extraction and gene expression analysis, was performed three times after exerting stress conditions (24h, 48h, and one week after treatment). These three sampling times are important stages in the early transcriptional response of plants to stress (Kong et al. 2015).

RNA isolation, cDNA synthesis and qRTPCR: Total RNA was extracted from samples using the TRIzol Reagent (Dena Zist Asia), and were treated with DNase I. Total RNA samples were used to synthesize first strand cDNA by manufacturer's instructions (cDNA synthesis, Takara kit). Real-time PCR was performed on QIAGEN's real-time PCR cycler, the Rotor-Gene Q. The forward and reverse primers for RT-PCR were designed from conserved domain of *Triticum aestivum* and *Hordeum vulgare* in NCBI database.

Results

The results revealed that the *PR1* gene was up-regulated in response to different treatments in both varieties, but the highest gene expression was observed under combination of drought and fungus treatment after one week and also under triple combination of drought, nematode and fungus treatment after 24h post inoculation in resistant variety to *F. culmorum* (2-49). Also nematode stress interaction with other stresses caused higher *PR1* gene expression in resistant variety to *H. filipjevi* (Silverstar). These up-regulations were significantly higher than gene expression under single stress conditions.

Our results revealed that *ERD15* gene was up-regulated in both varieties. Combined treatments such as drought/fungus, nematode/fungus and drought/nematode/fungus stresses caused the highest *ERD15* gene expression after one week in resistant variety to *F. culmorum* (2-49). Also interaction of nematode and fungus stresses caused extremely high gene expression in resistant variety to *H. filipjevi* (Silverstar) too.

Discussion

Plants have evolved some defense mechanisms to protect themselves from biotic and abiotic stresses. Cyst nematodes are biotrophic sedentary endoparasites of plants that can establish prolonged parasitic interactions with their host (Zhang et al. 2017), and *Fusarium* spp. are hemibiotrophs pathogens that incorporate both biotrophic and necrotrophic infection strategies (Ma et al. 2013). Plant resistance to biotrophic pathogens is classically thought to be mediated by SA pathway, and by contrast, resistance to necrotrophic pathogens is controlled by JA and ET pathways (Xiao et al. 2013). ABA is an important plant hormone that plays signaling roles in different processes of plant growth and development as well as in plant responses to various abiotic stresses like drought stress. ABA signaling pathway suppresses both SA and JA-ethylene signaling pathways. Hence, it is expected to activate several paths for resistance to multi stresses (Anderson 2004) (Figure 1 and Figure 2).

Results of present study showed that combined treatments either combination of two biotic stresses (*H. filipjevi* and *F. culmorum*) or combination of biotic (*H. filipjevi* and *F. culmorum*) and abiotic (drought) stresses caused a higher gene expression in wheat compared with single stress condition. In this regards, in roots of rice, simultaneous treatments such as *Meloidogynea graminicola*/methyl jasmonate and *Meloidogyne graminicola*/ethephon resulted in higher expression of *PR1* gene (Nahar et al. 2011). Arabidopsis plants under combination of biotic and abiotic stresses showed significantly less characteristic symptoms. Expression of *ERD15* gene was increased in Arabidopsis drought-treated plants by inoculation with *Paenibacillus polymyxa* and also co-inoculation of Arabidopsis plants by *Paenibacillus polymyxa* and *Erwinia carotovora* increased expression of *ERD15* (Timmusk et al. 1999).

Interestingly in the present study, combination of drought and each of pathogens attack caused a higher up-regulation in *ERD15* compared with up-regulation by each single pathogen stress. Salicylic acid (SA) is involved in the activation of systemic acquired resistance (SAR). The PR1 proteins which exhibit strong antifungal activity are often known as markers of the enhanced defensive state conferred by pathogen induced systemic acquired resistance (SAR). As mentioned above, *ERD15* enhances SA-dependent defense by antagonistic crosstalk with ABA signaling and therefore improves *PR* genes induction. In the present study, *PR1* gene showed a higher up-regulation under interaction of drought/pathogen stresses.

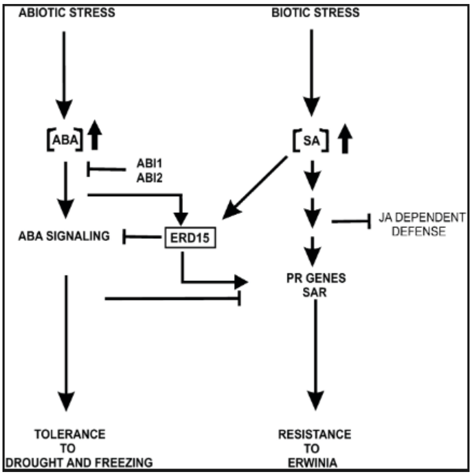

Figure 1. Hypothetical model presenting the role of *ERD15* in ABA responses. (By Kariola et al. 2006).

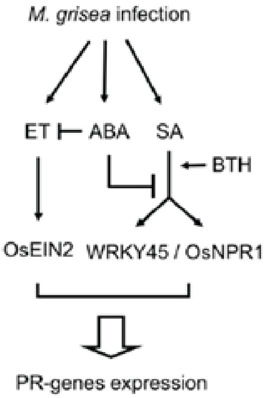

Figure 2. Schematic illustration of salicylic acid (SA)-abscisic acid (ABA), and ethylene (ET)-ABA antagonistic crosstalks in rice–*Magnaporthe grisea* interaction (By Jiang et al. 2010).

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Session VI
Novel approaches for designing cereal nematodes management strategies

Strategies to reduce plant parasitic nematodes damage on crops: a summary of literature with future prospect

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Introduction

Wheat (*Triticum* sp.) is among the most produced and consumed crops worldwide together with maize (*Zea mays* L.) and rice (*Oryza sativa* L.). Bread wheat (*Triticum aestivum* L.) is the most common cultivated crop around the world, which is mostly consumed as human food (FAO 2021). Population increase is not the only factor affecting food security and sustainability, stress factors are the major limitations in the production per unit area (Mahajan and Tuteja 2005; Reynolds and Borlaug 2006; Witcombe et al. 2008; Maccaferri et al. 2009; Tardieu and Tuberosa 2010). The current pace (<2% annual increase) does not meet the consumption (Borlaug 2002; Godfray et al. 2010). There is a continuous need for yield improvement due to increasing consumption and losses due to stress factors. Abiotic and biotic stresses have been estimated to cause 10 to 100% yield and product loss per year in combination with genotypic and other environmental factors (Reynolds et al. 2009; Schlenker and Lobell 2010). Major abiotic stress factors can be listed as drought, salinity, floods, radiation, heavy metals, and extreme temperature. On the other hand, various pathogens including fungi, bacteria, nematodes, and herbivores are the main biotic stress agents (Gull et al. 2019).

Biotic stress, which can be defined as the damage caused to a living organism by other organisms may limit plant production by up to 100% (Gull et al. 2019). Nematodes are one of the biotic stress agents and are responsible for an average of 10% yield loss worldwide (Seïd et al. 2021). There are more than 4000 different plant parasitic nematodes (PPN) currently identified (Seïd et al. 2021). PPNs are obligate biotrophic pathogens, they secrete effector molecules which make them parasitic organisms. PPNs significantly differ in the mechanism of parasitism and adaptation.

Most of the research focuses on the crop parasitic nematodes due to their effect on yield and overall productivity (Vieira and Gleason 2019). Nematodes have a wide range of host crops; they use almost all of the vegetable and cereal crops as host organisms. Some nematodes are species specific and have specific effectors and molecules, while others have wider host adaptation mechanisms and cross-species effectors (Vieira and Gleason 2019).

Common nematodes of wheat and their management

Root-lesion nematodes (RLN)

The most common genera of plant parasitic nematodes on cereals are Root-knot (*Meloidogyne* spp.), Cyst-forming (*Heterodera* spp.), Root-lesion (*Pratylenchus* spp.), Seed-gall (*Anguina* spp.), and Stubby-root (*Paratrichodorus* spp.) nematodes. Of these, root-knot nematodes (RKN), cereals cyst nematodes (CCN), and root lesion nematodes (RLN) are the major ones with the most significant impact on wheat production (Jones et al. 2013; Dababat et al. 2016). Even though RKN is the most common one, RLN and CCN are quite common and all three types of nematodes cause an estimated 125 billion USD loss worldwide (Keçici et al. 2022). Each PPN has a very specific host interaction. Fosu-Nyarko and Jones (2016) provide an in-depth analysis of RLN-host plant interaction mechanisms and potential control strategies using molecular genetic tools. One of the most effective strategies is to use resistant germplasm for PPN, if available. Mechanisms of resistance and tolerance to PPNs are genetically and independently controlled (Seïd et al. 2021). Resistance is the situation where, the host plant completely prevents nematode reproduction or damage of PPN to plant yield, while tolerance is the partial protection or control of PPN by the plant itself. On the other hand, susceptibility is termed as the inability of the plant to stop reproduction, spreading, and damaging of the PPN (Seïd et al. 2021). There are numerous studies reporting resistance/tolerance sources against various PPN species from wild or domesticated wheat gene pools. The soil-borne pathogen program at CIMMYT-Türkiye screens annually about 1000 genotypes from the International Winter Wheat Improvement Program (www.iwwip.org) under various conditions (growth room, greenhouse, and field) to PPN including *H. filipjevi*, *P. neglectus* and *P. thornei*. Zwart et al. (2004) reported five different synthetic wheat lines that can be utilized against RLNs (*P. thornei)*. They have suggested a polygenic resistance with additive gene action. Laasli et al. (2022) highlighted 48 lines resistant to *P. thornei* from 150 spring wheat germplasm, while Thompson et al. (2012) also reported resistance sources against RLNs *P. thornei* and *P. neglectus* in five different double haploid populations of wheat. They highlighted a quantitatively inherited resistance mechanism. Novel resources for RLN resistance in Turkish wild emmer (*T. dicoccoides*) were reported by Toktay et al. (2015). Their results indicated a total of 17 lines with improved or equal resistance against *P. thornei* and *P. neglectus* compared to check lines. Several QTLs were reported by Rahman et al. (2020) for resistance against *P. thornei*. Two major QTL on chromosomes 2B (QRlnt.sk-2B.1–2B.3) and 6D (QRlnt.sk-6D.1 and 6D.2) were responsible for 30 and 48% of the genotypic variation, respectively. Thompson et al. (2009) screened Watkins (148 bread and 139 durum wheat accessions) and the McIntosh Collections (59 bread and 43 durum wheat accessions) for resistance against *P. thornei*. The results indicated that 13 bread and 10 durum wheat accessions had a similar tolerance level (low nematode numbers) similar to GS50a, a partial resistant reference wheat line. Thompson (2008) detected lines resistant to RLN from a synthetic hexaploid population when compared to the partially resistant control line GS50a. In addition, new sources for resistance to RLN in *T. durum* and *Aegilops tauschii* were obtained (Thompson et al. 2008). In a study on wheat wild relatives and progenitors, 251 accessions were screened from wheat wild relatives (*A. speltoides*, *A. peregrina*, *T. urartu*, *T. monococcum*, *T. timopheevii* ssp. *armeniacum*, *T. carthlicum*, *T. dicoccoides*, *T. turanicum*), hexaploid wheat with disease resistance introgressed from *A. tauschii*, *A. peregrina* carrying two CCN resistance genes (CreX and CreY), Iraqi landraces and doubled haploid lines derived from the GS50a. A significantly higher number of resistant lines were reported in the diploid wild relatives of wheat, compared to tetraploid ones (Sheedy et al. 2012). In another screening study, a total of 274 Iranian landraces were evaluated for resistance against *P. thornei* (RLN) and 25 landraces from Iran were reported to be more resistant than GS50a (Sheedy and Thompson 2009). An in-depth evaluation of the nematode resistance mechanism was evaluated by Dababat et al. (2016). Accordingly, a total of 126 CIMMYT advanced spring wheat lines were screened for resistance to *Heterodera avenae*, *P. neglectus*, and *P. thornei*, of which 107 lines were genotyped with 1,310 DArT markers. It was confirmed that chromosome 4A (~90–105 cM) can be a source of resistance to *P. thornei*. Several novel QTLs were mapped on chromosomes 5A, 6A, and 7A for *H. avenae* (CCN); on chromosomes 1A, 1B, 3A, 3B, 6B, 7AS, and 7D for *P. neglectus* (RLN); and on chromosomes 1D, 2A, and 5B for *P. thornei* (RLN) (Dababat et al. 2016)**.** A Genome-Wide Association Study (GWAS) was carried out by Sohail et al. (2022) for resistances to *Pratylenchus* sp. and eleven different single nucleotide polymorphisms (SNPs) on chromosomes 1A, 1B, 2A, 3A, 4A, 5B, and 5D were significantly associated with RLN resistance. In a similar GWAS study, Pariyar et al. (2016) screened 161 winter wheat accessions with a 90K iSelect SNP chip. Accordingly, eight QTLs on chromosomes 1AL, 2AS, 2BL, 3AL, 4AL, and 5BL were linked to putative genes known to be involved in plant-pathogen interactions. In a study, Dababat et al. (2019) found that 56 lines from the CIMMYT spring wheat collection (484 lines) were resistant to *P. thornei* under controlled conditions. When these 56 lines were examined in terms of resistance/tolerance under field conditions, it was seen that 14 lines maintained their resistance and 16 lines were moderately resistant, and four lines had full resistance. Even though the above reports identified a pool of tolerance/resistance alleles or genetic resources for PPNs, there is a necessity for continuous evaluation of germplasm resources, wild and domesticated accessions, for the continuous fight with current and future PPNs.

Cereal cyst nematodes (CCN)

Another group of PPN is cereal cyst nematodes (CCN), *Heterodera* spp., which cause significant yield losses in wheat. To cope with CCN, there is a continuous effort for identifying resistance sources. For this purpose, at least nine single dominant resistance genes (*Cre* genes) have been reported, many of which were derived from wild relatives of wheat (Dababat et al. 2015). Six *Cre* genes (*Cre*2 to *Cre*7) were derived from *Aegilops* spp. (Jahier et al. 2001); other resistance genes were derived from *T. aestivum* (*Cre*1 and *Cre*8) and rye (*Secale cereale*) lines (*Cre*R) (Barloy et al. 1996). Singh et al. (2010) evaluated *T. monococcum* (AA genome) lines against CCN and mapped QTL in a population created using a resistant *T. monococcum* line. Two QTL on chromosomes 1A and 2A were reported to be responsible for the resistance mechanism with 26% and 13% phenotypic variance, respectively. A GWAS study identified resistance to *H. filipjevi* in wheat genotypes by the presence of 11 QTLs on different chromosomes (Pariyar et al. 2016). Another study screened 188 wheat genotypes received from ICARDA collection originating from seven countries using a 152K SNP chip. They reported significant differences among the genotypes for resistance against *H. filipjevi* (Majd Taheri et al. 2019). *Aegilops triuncialis* and *A. tauschii* were reported to have genes for the resistance against CCN (Romero et al. 1998). Cui et al. (2020) evaluated perennial wheat lines generated from a cross between common wheat (*T. aestivum*) or durum wheat (*T. turgidum* var. *durum*) and the intermediate wheatgrass (*Thinopyrum intermedium*). According to their results, perennial wheat was completely resistant to *H. avenae* and *H. filipjevi*, which is thought to be due to alien chromosomes from the *Th. intermedium*. Vishnudasan et al. (2005) reported a transgenic approach to CCN (*H. avenae*) infestation. They engineered a *plant serine proteinase inhibitor* (*pin2*) gene into *T. durum* and obtained a significant level of resistance against *H. avenae*.

Management methods

There is a need for the continuous management of the stress factors with genetic improvement, field applications, and biological and chemical control agents for sustainable and sufficient food production (Hall and Richards 2013; Meister et al. 2014; Langridge and Reynolds 2015; Onaga and Wydra 2016; Vurukonda et al. 2016; Li et al. 2020). The most common and practical method for the control of nematodes is the use of chemical agents. However, chemical control with the nematicides is not the environmentally safest way, since they pollute the soil and water, and negatively affect the entire rhizosphere (Alengebawy et al. 2021). Biological control is another way to cope with nematodes, which is still a developing subject (Stirling 2018; Molinari and Leonetti 2019; Poveda et al. 2020; Ahmad et al. 2021). Nematicides are still the most common nematode control method available. Sasanelli et al. (2021) and Seïd et al. (2021) reported various types of PPN control methods including but not limited to; fumigant and non-fumigant nematicides, agronomic practices (crop rotation, biofumigation, cover crops, fallow, trap crops, soil amendments, etc.), physical methods (soil solarisation), Biopesticides (fungal, bacterial, and bacterial derived based), and several plant extracts (garlic extract, clove oil, thymol, etc), as the nematode control methods in the field. Each of these management methods has pros and cons based on application dose, application time, field conditions, plant and nematode species, and soil conditions, however, the economical limitations and some requirements for advanced expertise should not be overlooked. The long-term strategy to deal with PPN as a more widely applicable method and an inevitable priority is to select/breed "PPN resistant" cultivars. Moreover, environmentally friendly soil applications and soil amendments by using bio-fertilizers and biological control agents need more attention and support from the international research community. Since complete elimination of nematicides is not possible in the short term, reducing their use is necessary for the agricultural community to approach a sustainable and environmentally friendly PPN control strategy.

Future prospects

Even though there is a large number of studies that reported screening, defining, and finding resistance/tolerance sources, in the end, there is still no sufficient and global method available for the control of PPNs. Plant parasitic nematodes still cause significant yield losses in the wheat fields and farmers are mostly obligated to apply nematicides. Genetic characterization studies including QTL analyses, GWAS studies, genomic selection, other mapping approaches, gene identification, etc. are numerous. However, very few of those turn into real-life solutions against PPN in the field. It is no doubt that, without pre-screening, there is no pre-breeding material. Most of the common studies screen populations or germplasm panels to identify genetic loci associated with PPN resistance, while very few reports have validated or gene-associated results. This makes most of the QTL identified in the research "a hard to use" material for the actual plant breeders. A brief literature search brings dozens of resistance screening publications, when it comes to reporting resistant "cultivars" the numbers turn into "a few". There is an urgent need for the identification, validation, or breeding of cultivars with moderate to complete resistance against PPNs. Marker-assisted selection, GWAS, and other biotechnological approaches should be utilized in combination with the classical breeding methodologies. Research should have more focus on end-user products, than screening. More support should be directed to "result-oriented" projects than pure screening approaches.

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The CAIGE (CIMMYT-AUSTRALIA-ICARDA GERMPLASM EVALUATION): the Concept, and its integration of Soil Borne Resistance into high yielding adapted germplasm for Australian and Global Wheat Improvement.

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Abstract

The CAIGE (CIMMYT-AUSTRALIA-ICARDA Germplasm Evaluation) concept has proven to be an effective way for Australian Cereal pre-breeders and pathologists optimize the use of wheat genetic resources from the two International Research Centers including CIMMYT and ICARDA which are part of the CGIAR. Funded by the Australian Grains Research and Development Corporation this multi-partner collaborative program coordinates the selection, importation, quarantine, multiplication and national assessment of the international crop germplasm (bread wheat, durum wheat and barley) in Australia, and manages and disseminates the information generated. CAIGE aims to extract as much value of relevance to local Australian scientists and pre-breeders as possible to optimize decisions on germplasm choice and use. Valuable germplasm has been identified for the main cereal cropping regions of Australia which is high yielding and adapted as well as having valuable sources of abiotic (drought/heat) and biotic (various foliar and Soil Borne Pathogens) resistances and tolerances. In collaboration with the CIMMYT-Turkey program and Australian pathology partners this project has been able to identify some valuable sources of genetic resistance to key Soil Borne Pathogens (Crown Rot (*Fusarium psuedograimearum* and *F. culmorum*), and Root Lesion Nematodes (*Pratylenchus thornei* and *P. neglectus*)) in both bread and durum wheat. These sources may also prove to be useful for other programs around the world, as may the CAIGE model of germplasm exchange and collaboration.

Introduction

Importance of Soil Borne Diseases – Root Lesion Nematode and Crown Rot to Australian Cropping Systems.

Like many other large rainfed wheat production systems Australia also has significant economic yield loss due to Soil Borne Pathogens. These includes the Root Lesion Nematodes - RLN (*Pratylenchus thornei* and *P. neglectus* predominantly) and also the fungus Crown Rot - CR (*Fusarium psuedograimearum* and *F. culmorum*).

The RLNs, *P. thornei* and *P. neglectus* are globally important pathogens of both cereal (bread and durum) and legume crops, and often occur together in many farming systems. They have been reported to cause significant yield losses up-to 60% on wheat both in Australia (Nicol *et al.* 1999, Taylor *et al*. 1999, Thompson *et al.,* 2021) and globally from Mexico (Nicol & Ortiz-Monasterio 2004a), USA (Smiley *et al*. 2008) and several parts of West Asia and North Africa (Hajihasani *et al*. 2010, Nicol *et al.* 2004b). Former economic analysis by Murray and Brennan (2009) puts a value of Australian $199M /year for nematodes on wheat systems in Australia.

As reviewed by Sheedy *et al*. (2022) many quantitative trait loci (QTLs) for *P. thornei* resistance have been reported in wheat, and similarly several studies have reported QTLs associated with *P. neglectus* resistance. However, the QTLs often explained only a low proportion of genetic variation, or they required validation in a genetically diverse panel or appropriate breeding populations before being suitable for use in marker-assisted selection (MAS). Presently there is limited use of effective markers for RLN in Australian breeding programs.

CR caused by Fusarium crown rot caused by *Fusarium culmorum* (W. G. Sm.) Sacc. and *F. pseudograminearum* (Aoki and O'Donnell; group I) (=*Gibberella coronicola* can cause high yield losses on wheat in dryland areas with drought conditions worldwide (i.e. rainfed wheat production systems or reduced irrigation) (Burgess *et al*. 2001, Cook 1980 & 1992, Chekali *et al.* 2011, Hollaway and Exell 2010, Nicol *et al.* 2007, Paulitz *et al*. 2002, Papendick and Cook 1974). Durum wheat (*Triticum durum*) is known to be more intolerant (suffer greater yield loss) than bread wheat (Chekali *et al.* 2011, 2013). A recent study of the economic impact of CR in Australia, based on inoculum levels in 5,993 fields (2015 to 2017) determined using qPCR molecular analysis (PREDICTA®B), estimated average annual losses of 6% (Australian $404 million) with losses of 8-10% possible across the wheat cropping regions (Hollaway *et al*. 2022). CR disease is increased in importance with the widespread adoption of reduced tillage, stubble retention practices and intensification of cereals within cropping systems (Burgess *et al.* 2001, Evans *et al*. 2010, Paulitz *et al*. 2002, 2006). Unlike RLN legumes are not known hosts to CR so appropriate rotations are part of an integrated management strategy with cereals Inter-row sowing can dramatically reduce CR damage, which allows farmers to take full advantage of reduced tillage. (Simpfendorfer 2012). Management of CR is difficult due to limited tolerance and resistance in commercial wheat cultivars (Davies pers. comm, Ozdemir *et al.* 2020, Thompson *et al.* 2020, Vanstone *et al.* 2008) and a lack of registered effective chemical options.

Previously in Australia the Cereal Cyst Nematode – CCN (*Heterodera avenae* pathotype *Ha13*) was also a major economic constraint in the rainfed stressed wheat production systems of the southern region, however this was resolved with the integrated research efforts of geneticists, pathologists and breeders (including molecular biologists) where a number of major gene resistant sources were identified and incorporated into Australian commercial varieties (Vanstone *et al.* 2008). Even now these sources are continued to be used and confirmed with molecular tools in the corporate sector breeding programs. This is a story of great success in pathology and breeding in Australia in part due to the excellent coordinated research and the fact that only one species and pathotype of CCN is present (unlike many other parts of the world, Nicol *et al.* 2004b, Toumi *et al*. 2018).

The challenge with RLN and CR is that the resistance (ability of the plant to reduce the multiplication of the pathogen) and tolerance (ability of the plant to yield despite attack of the pathogen) is complex and is controlled by several minor genes. This requires a large effort in exploiting genetic resistance/tolerance and pyramiding this into high yielding adapted wheat lines. Another significant challenge is that frequently in rainfed water stress wheat predominated production systems of the world these Soil Borne Pathogens occur together as a complex (Nicol *et al.* 2007, Şahin *et al.* 2008, Sheedy *et al*. 2022, Thompson *et al.* 2010, Tunali *et al.* 2008, Yavuzasanoglu *et al.* 2012), and an approach of multiple disease resistance breeding should be considered (Nicol *et al*., 2007).

With the advances in molecular biology Australia has pioneered the development of the pre-plant qPCR soil/stubble test (PREDICTA®B) provides growers with a valuable tool to identify fields at risk of loss due to CR, RLN and CCN and implement available integrated management strategies (Hollaway *et al.* 2013). PREDICTA®B is also a great research tool for monitoring and assessing SBP and other pathogens for researchers.

The CAIGE project offers a great opportunity to exploit the genetic diversity for both SBP resistance and tolerance (and other abiotic and biotic constraints), and with the coordinated efforts of plant pathologists, pre-breeders and breeding programs offers the opportunity to identify, pyramid and combine these genetic sources into high yielding adapted lines for use by the Australian wheat improvement sector and global wheat improvement programs such as CIMMYT and ICARDA and their valued National Program partners. Australia has been working actively in this area, especially for RLN and CR for some time and work has identified many sources of genetic resistance for RLN and significant progress has been made to identify the novelty of these genes and pyramid them (Sheedy *et al*. 2022, Davies *et al. pers.comm*). Presently only a limited number of sources are used in commercial breeding programs and some of these have reliable markers to enable them to be selected along the breeding pipeline (Sheedy *et al*. 2022, Davies *et al. pers. comm*.).

It is important to note that genetic resistance is part of the solution to help control these complex soil borne pathogens of CR an RLN, however effective control required an integrated management approach of which resistance is one component and crop rotation, possible seed treatments and a sustainable production system (with optimal soil health) will ultimately provide effective control of these pathogens (Evans *et al*. 2010, Owen *et al*. 2022, Simpfendorfer *et al*. 2019, Vanstone *et al.* 2008).

Materials and methods

The CAIGE Concept

The CIMMYT-Australia-ICARDA Germplasm Evaluation (CAIGE) program was established in 2006 in Australia to better exploit genetic diversity in wheat (bread and durum) and barley sourced from the Consultative Group on International Agricultural Research (CGIAR) for wheat improvement in Australia. This collaborative program coordinates the selection, importation, quarantine, multiplication and national assessment of the international crop germplasm, and manages and disseminates the information generated. CAIGE aims to extract as much value of relevance to local scientists and farmers as possible to optimize decisions on germplasm choice and use (Figure 1.). Partners include universities, government organizations, commercial entities and wheat breeders of the CGIAR, and is supported by the Australian Grains Research and Development Corporation (GRDC).

All CGIAR data linked to the imported germplasm and data captured on the same materials in Australia are available to all partners via the CAIGE website (caigeproject.org.au) and database. The CGIAR data is used to hone selections made by Australian crop breeders at each of the centers in alternate years. Original seeds delivered to Australia are grown and screened for pest and disease by the Australian Grains Genebank (AGG) and then multiplied post-quarantine at Narrabri before being distributed nationally in multi-environment yield trials. The materials are concurrently screened for disease resistance and some grain quality traits by Australian collaborators. A subset of materials is also screened for their resistance to the important root diseases on wheat. Most recently in the new reiteration of CAIGE the Yield Trial sites have been assessed with PREDICTA®B to establish the background levels of SBP (CR and RLN), so more attention can be paid to the performance of the genetic materials in those locations, particularly if the wheat crop is under water stress.

In partnership with CBADS-SPI (Centre for Biometrics and Data Science for Sustainable Primary Industries) at the University of Wollongong Australia, recent advances in statistical methodology are used to both design and analyze yield trials. Currently, a rolling multi-environment (four year) pedigree-based Factor Analytic Model is used for all three crops. The incorporation of iClass and CAIGE SHINY APP (an interactive data visualization tool) in data analysis has enhanced breeder decision making.

Underpinning all of these, and in collaboration with the International Breeding Platform (IBP), the CAIGE pedigree and phenotypic data are stored in an application of the Breeding Management (BMS) system, which enables collaborators to search and extract relevant data. BMS also provides a platform for GIGWA which stores relevant genotypic data. The CAIGE program has successfully improved the uptake and use of these globally important materials for the benefit of Australian grain growers.

Results and discussion

Progress to date with the validation of genetic resistance for CR and RLN

Since 2012 concerted efforts screening a subset of CAIGE germplasm and sharing SBP data from CIMMYT-Turkey and Australia for CIMMYT germplasm have resulted in a number of lines (greater than 15) with confirmed resistance to one or both species of RLN and/or CR. These lines are in high yielding adapted backgrounds and offer potentially new sources of resistance to the breeding community, both in Australia and internationally. Furthermore, these lines also have several other valuable biotic resistances for foliar pathogens (such as rust, yellow spot and Septoria). Further work is necessary to further validate these sources and determine if their resistances to these SBP are novel.

One of the outputs of the CAIGE project is to provide collaborators with a 'key disease core' set which provides the valuable lines for resistances to one or more pathogens. This is a public resource which can be shared with the Wheat Improvement Community. This information can be found on the CAIGE website and will be updated every 3 years as the CAIGE project progresses.

This paper demonstrates through coordination, collaboration and integration of the key disciplines of pre-breeding and pathology how valuable sources of resistance and yield can be used by the wheat improvement community and ultimately farmers in the form of new varieties or cultivars. This model may also serve useful for other wheat improvement programs.

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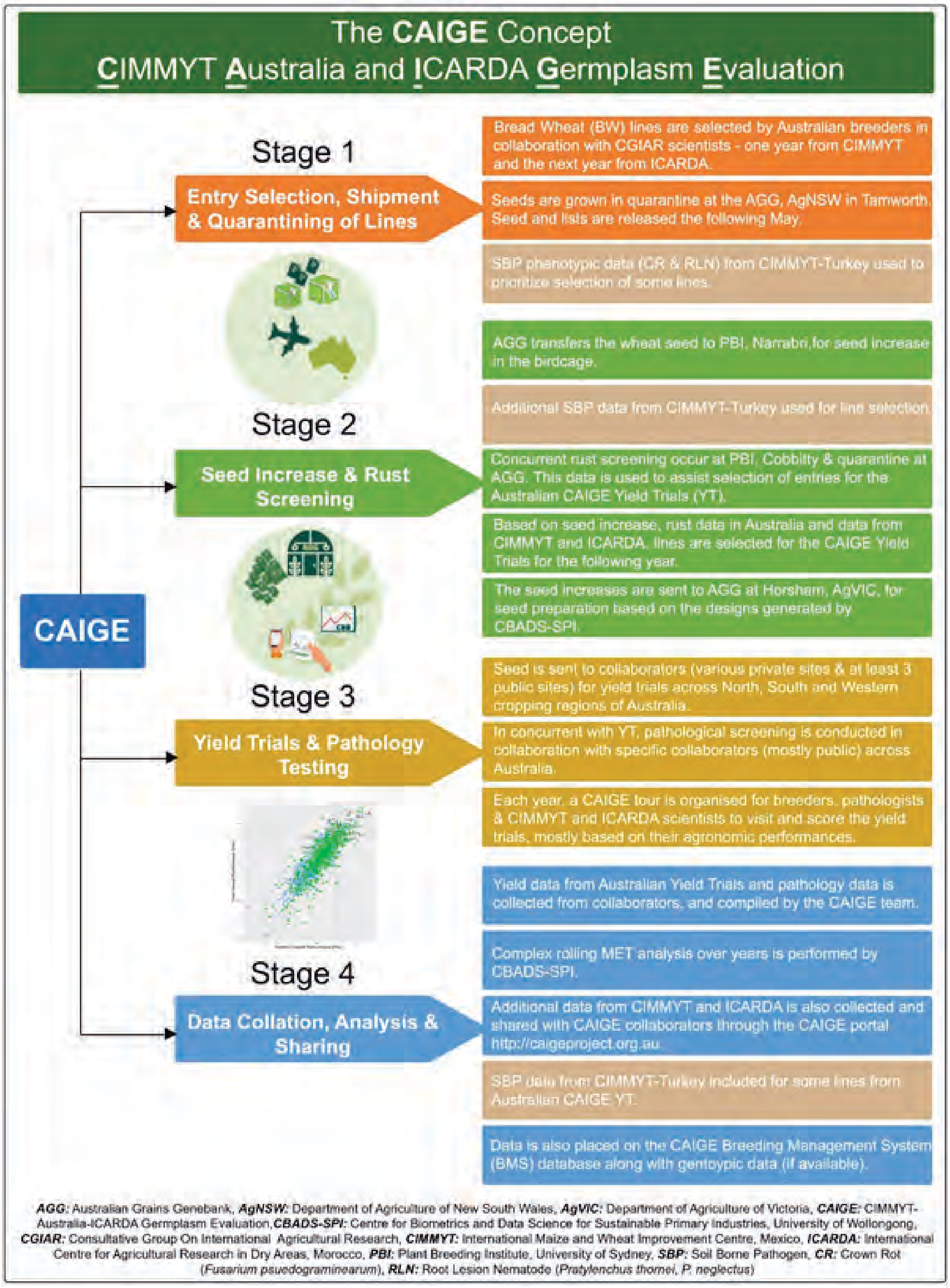

The **CAIGE** Concept
CIMMYT Australia and ICARDA Germplasm Evaluation

CAIGE

Stage 1

Entry Selection, Shipment & Quarantining of Lines

Bread Wheat (BW) lines are selected by Australian breeders in collaboration with CGIAR scientists - one year from CIMMYT and the next year from ICARDA.

Seeds are grown in quarantine at the AGG, AgNSW in Tamworth. Seed and lists are released the following May.

SBP phenotypic data (CR & RLN) from CIMMYT-Turkey used to prioritize selection of some lines.

Stage 2

Seed Increase & Rust Screening

AGG transfers the wheat seed to PBI, Narrabri,for seed increase in the birdcage.

Additional SBP data from CIMMYT-Turkey used for line selection.

Concurrent rust screening occur at PBI, Cobbity & quarantine at AGG. This data is used to assist selection of entries for the Australian CAIGE Yield Trials (YT).

Based on seed increase, rust data in Australia and data from CIMMYT and ICARDA, lines are selected for the CAIGE Yield Trials for the following year.

The seed increases are sent to AGG at Horsham, AgVIC, for seed preparation based on the designs generated by CBADS-SPI.

Stage 3

Yield Trials & Pathology Testing

Seed is sent to collaborators (various private sites & at least 3 public sites) for yield trials across North, South and Western cropping regions of Australia.

In concurrent with YT, pathological screening is conducted in collaboration with specific collaborators (mostly public) across Australia.

Each year, a CAIGE tour is organised for breeders, pathologists & CIMMYT and ICARDA scientists to visit and score the yield trials, mostly based on their agronomic performances.

Stage 4

Data Collation, Analysis & Sharing

Yield data from Australian Yield Trials and pathology data is collected from collaborators, and compiled by the CAIGE team.

Complex rolling MET analysis over years is performed by CBADS-SPI.

Additional data from CIMMYT and ICARDA is also collected and shared with CAIGE collaborators through the CAIGE portal http://caigeproject.org.au

SBP data from CIMMYT-Turkey included for some lines from Australian CAIGE YT.

Data is also placed on the CAIGE Breeding Management System (BMS) database along with gentoypic data (if available).

AGG: Australian Grains Genebank, ***AgNSW:*** Department of Agriculture of New South Wales, ***AgVIC:*** Department of Agriculture of Victoria, ***CAIGE:*** CIMMYT-Australia-ICARDA Germplasm Evaluation,***CBADS-SPI:*** Centre for Biometrics and Data Science for Sustainable Primary Industries, University of Wollongong, ***CGIAR:*** Consultative Group On International Agricultural Research, ***CIMMYT:*** International Maize and Wheat Improvement Centre, Mexico, ***ICARDA:*** International Centre for Agricultural Research in Dry Areas, Morocco, ***PBI:*** Plant Breeding Institute, University of Sydney, ***SBP:*** Soil Borne Pathogen, ***CR:*** Crown Rot (*Fusarium psuedograminearum*), ***RLN:*** Root Lesion Nematode (*Pratylenchus thornei, P. neglectus*)

Figure 1: The CAIGE (CIMMYT and ICARDA Germplasm Evaluation) Concept Model for Wheat Exchange and Improvement. The boxes in yellow show the integration of Soil Borne Pathogen (SBP) resistance.

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Session VII
Breeding for Soil Borne Pathogens

Efficient cereal breeding to bridge the gaps for better SBPs management

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Introduction

Losses from plant parasitic nematodes are major constraints for crop production, annual global losses for all crops are estimated 78$ billion (Siddique and Eves-van den Akker 2022) i.e. at least 10% of the total global crop value are lost due to nematodes. Savary et al. (2019) estimated that current yield losses of 21% in wheat are due to pests and diseases. Estimates for yield losses from cyst and lesion nematodes in wheat range from 10-100 % (Seid et al. 2021); for N-Europe, Dababat and Fourie (2018) report 10% annual losses from cyst nematodes alone. Totaling losses for other Soil Borne Diseases (SBD) caused by fungi, viruses and bacteria, SBD are likely to cause economic losses comparable or in some cases higher than other above ground plant diseases and pests. By any standards, these substantial losses would warrant major investments in developing resistant and in case of nematodes also tolerant varieties. However, breeding for resistance or tolerance to nematodes and soilborne diseases, have not been made a priority in wheat breeding programs, apart from few exceptions in Australia, where root lesion nematodes resistant wheat varieties were released and new breeding lines with tolerance to crown rot were developed (Trethowan 2021).

In theory it is known what is required to control Cereal Cyst Nematodes. Smiley (2022) stated: “Production of cultivars which are both resistant and tolerant will become the most effective method for controlling CCN. Resistant and tolerance are genetically independent, and all combinations of resistance and tolerance are possible within a collection of cultivars (Smiley, 2022)”. In reality however, there is very little research and even less breeding work on SBD in wheat, in particular in countries of the South. CIMMYT’s SBD program in Türkiye is an exception, though even this program has focused on screening germplasms to identify new sources of resistance, surveys and yield loss trials but focused less on developing elite lines with tolerance/resistance to SBD. This paper discusses some aspects, why breeding for resistance to SBD is so challenging.

Breeding for SBD in wheat – a major challenge

There is a multitude of reasons for this. First of all, soil borne diseases can be found in many wheat fields, but the damage causing organisms are not easily detected and symptoms are often not obvious and can easily be confused with other problems, e.g. nitrogen deficiency or drought stress. In other words, there is no awareness that SBD as a constraint for wheat production and even less for the economic losses. For example, diagnosing the CCN species and then the dominant pathotype – the equivalent of races in cereal rusts - is essential for a meaningful breeding intervention, as this defines which genes should be introgressed, but this information is often not available. In breeding for cereal rust resistance, detecting the dominant and monitoring occurrence of new races is paramount to develop resistant wheat varieties. Dababat (pers. Comm) has conducted surveys in Central and West Asia and North Africa and found crops severely suffering from various SBD in most of the rainfed wheat systems, while there was a general lack of awareness for the losses due to SBD. Once SBD have been recognized and confirmed as a constraint, yield loss trials are usually conducted. These trials indicate generally substantial losses greater 20%. A frequent shortcoming of these trials is that they are conducted in selected fields with a previous record of high infestation or even in artificially inoculated sick plots and do not necessarily represent the conditions in farmers' fields. In short, SBD cause serious yield losses in wheat and often more than one SBD is present and different diseases are dominant over years. Too often, there is not enough information available to help breeders decide on which disease to focus. Since every trait added to the core trait list of a wheat breeding program slows down the genetic gain rate of the other traits, sound data must be produced to justify inclusion of a SBD into a wheat breeding program.

Genetic variability as basis to breed for resistance to Soil Borne Diseases

Though losses from SBD in wheat are economically substantial and in cases where breeders are aware of the dominant SBD within their region, breeding for resistance to SBD has not been prioritized, apart from a few exceptions, in particular for nematodes and crown rot. SBD pathologists have screened a huge number of wheat accessions, both elite lines and genetic resources and have identified lines with variable levels of resistance / tolerance. However, the reports refer generally to field and lab scores, but often fall short of providing information regarding the genetics, which is paramount to initiate a successful breeding program. The issue is further complicated by the fact that sources of resistance are often in a poor agronomic background and breeders generally stay away from using agronomically poor lines for crossing. The introgression of resistance from unimproved sources with known genetic make-up is best addressed through a dedicated pre-breeding program, where the focus is on transferring the trait of interest into an elite line. However, today there are very few pre-breeding programs operating, and most of those focus on other traits than SBD. A successful example is breeding for resistance to an extremely challenging disease like crown rot, where the known resistance is highly quantitative and many chromosomal regions carrying minor resistance genes need to be combined. Trethowan et al. (2021) have combined 8 regions with resistance genes and through marker assisted recurrent selection pyramided 48 minor genes. This was achieved at the end of a ten-year project using all available most recent technologies and illustrates the significant investments in time and staff needed to tackle such difficult diseases.

An obvious but often unrecognized reason is that breeders do not prioritize breeding for SBD resistance, due to the lack of genetic variation and lack of knowledge regarding the genetic make-up of a particular resistance. A major exception is CCN. Seid et al (2021) report that nine resistance genes (*Cre*1–*Cre*9) to CCN are well-documented in the literature. *Cre*R, *Cre*V and *Cre*Z genes are relatively recently characterized from wheat and confer resistance to CCNs. But for lesion nematodes, Rlnn1 is the only resistance gene characterized from wheat that is known to confer resistance towards RLN. A similar situation, i.e. no resistance genes were identified, exists for other important SB fungal diseases such as wheat common root rot (*Bipolaris sorokiniana*), Fusarium crown rot (*Fusarium pseudograminearum* and other *Fusarium* pathogens including *F. culmorum*, *F. avenaceum*, and *F. graminearum)* sharp eyespot sheath blight, (*Rhizoctonia cerealis*), and take all (*Gaeumannomyces graminins var. tritici).* Only a few wheat genotypes show moderate resistance to these root and crown rot fungal diseases, and the genetic determinants of wheat resistance to these devastating diseases are poorly understood.

A further complicating factor are the significant differences between plant resistance to root pathogens and to foliar pathogens (Zhang et al. 2020). Therefore, it is necessary to investigate the responses of plant roots to root pathogens and the corresponding defence mechanisms rather than just rely on results of plant leaves to foliar pathogens. Defense mechanisms of plants against SBD that are understood, are highly complex and suggest that resistance is highly quantitative. These highly quantitative traits are also an indication, that single gene solutions are unlikely to be found. If they existed in elite lines, they had been likely detected. Most of the wild relative accessions stored in gene banks still wait to be screened for resistance to SBD and other diseases as well. Recognizing that SBD for wheat are likely to become more important in the future, since the low value crop wheat is likely to be pushed into more marginal areas, where a healthy root system is essential to cope with the abiotic and biotic stresses, it is paramount that more attention is given to developing wheat varieties with better tolerance / resistance to SBD.

Breeding options

Smiley (2022) stated, that *Cre*1 provides best resistance to CCN, but suggests pyramiding *Cre*1 with other *Cre* genes to enhance the resistance. This breeding approach is feasible, when good or perfect markers are available, since otherwise it is very difficult to identify the genotypes that carry both genes to be pyramided, when the genes are dominant. Pyramiding genes is a concept used in breeding wheat rust resistance for decades, but due to the difficulties to combine independently segregating alleles, whose effects are not easily detectable, only very few breeding programs applied this breeding strategy. CIMMYT wheat breeders used this approach, i.e. combining genes with minor resistance effects, since the 70s very successfully. But this approach requires long term commitment and funding. A major factor was, that morphological markers could be identified (e.g. tip necrosis is linked with *Lr* 34, a gene that provides partial leaf rust resistance) that allowed to successfully make phenotypic selections. Progress became faster, when molecular markers became available, as this also allows now to combine major genes. But even then, pyramiding genes, whether recessive of dominant, remained a major challenge for breeders, since many markers are not perfect and developing markers that are reliable across genotypes with different background remains a major task.

It is a common observation, that QTLs are identified and published, also for SBD, but the next step, developing and verifying a marker in different genotypes, is not taken, since this is often a time-consuming process with limited scientific credit. An important point to consider is that if both parents do not carry the resistance / tolerance genes, these genes segregate and need to be recombined in every new cross. Without reliable markers, effective selection is a major challenge. In the absence of markers, one approach could be to use Single Seed Descent (SSD) or Speed Breeding, i.e. plants are advanced with minimal selection pressure to a homozygous stage (F5 or F6) and are then evaluated for resistance by plant pathologists.

For diseases agents like CCN, where specific resistance genes have been identified, gene stacking would be a powerful approach. The advent of rapid cloning technologies has facilitated the development of stacks with up to 8 rust resistance genes (Luo 2021). While current focus is on stem rust, this approach can be expanded to any other wheat diseases, including SBD, provided the cloned genes are available. The technology used to stack the genes is considered genetic modification (GM), even though the genes used originate from wheat and wheat relatives, i.e. cis-genic. Work is underway, to use genome editing to develop gene stacks, as this technology is not considered GM in some countries.

A solution exists now to basically curtail the most important wheat diseases. As a next step, once the optimal – non segregating - stacks have been identified, they can be transferred among others into CIMMYT's elite lines and distributed internationally for global use, provided policy makes allow use of GM technology and consumers accept the product. Due to COVID-19, the world has been alerted to the risks caused by new diseases and this will hopefully affect the position law makers and consumers take.

Hubs for SBD screening

To screen new wheat lines for resistance / tolerance to SBD it is essential that the material can be reliably evaluated on an annual basis in the lab and in the field. This requires the establishment of sick plots and access to labs that are equipped to handle material infested with a particular SBD and the operation is managed by a SBD pathologist. Since very few wheat breeding programs have access to such an infrastructure and / or work closely with SBD pathologists, a more efficient approach is to set up hubs at a hot spot for a particular disease or abiotic stress. At these hubs not only lines from the host program are tested but interested cooperators can submit their material for testing. The data produced for all lines are published. Such hubs have been established and are supported by CRP WHEAT of the CGIAR, BGRI/Cornell and IWYP. Hubs are operating for Fusarium head blight (FHB) in China; FHB, leaf rust and Septoria in Uruguay; wheat blast in Bangladesh and Bolivia; heat and drought in Mexico and Morocco; yield potential in Mexico, USA, and UK; and for stem and yellow rust in Kenya. For SBD – CCN, RLN and CR – a hub is operating in Türkiye. Using this hub with infrastructure and dedicated staff assures that co-operators get reliable data for the submitted lines as basis for effective annual selection. Breeding programs have thereby information about the level of resistance / susceptibility in their material they otherwise would not get. Though these hubs have proven to be very effective and cost efficient to screen large numbers of lines, their long-term funding is not assured.

Breeding for SBD resistance without knowledge of the underlying genetic system

As pointed out above, for many SBD there are no good resistance sources known resp. the genetic system for resistance is not well understood. In this paper Integrated Pest Management / Integrated Nematode Management is not discussed. But it is well understood that for diseases which can't be effectively controlled through resistant varieties, IPM/INM measures are the best, only and very effective option, in particular when nematicides are not released or available as is the case in many countries in the South. The challenges and success of this approach are well documented (Sikora et al. 2022, Simon et al. 2021) and Helder and Heun (2022) point rightfully out, that durable plant parasitic nematode management is becoming much more knowledge intensive than in the past.

Breeding highly resistant varieties has not been achieved for most SBD in wheat due to lack of resistance genes and / or sufficient investments, but varieties with variable levels of susceptibility / resistance have been identified. Where success has been reported (Trethowan et al. 2021, Fusarium Crown rot, see above), major investments over a decade were needed, going far beyond what is available to most breeding programs; and the challenge for breeders to introgress the highly polygenic resistance into elite lines remains. Such breeding approaches require a strong interaction between breeders, pathologists, geneticists, and statisticians, which few breeding programs in the South can build on.

Though for many SBD no complete control through resistant varieties is possible, it is paramount that in regions where SBD are known to be important, lines entering yield trials are screened for their tolerance to the prevalent diseases and susceptible lines are discarded. The release of "disease suckers" should be strictly avoided. Such a regular screening would be a big contribution to make IPM more effective, and over time the level of tolerance in the breeders' elite line should increase. This information should also be publicly available to farmers can make a better decision re variety. Hubs as described above, are ideal to provide this information.

Conclusion

Soil borne diseases in wheat, though a major constraint for production, are often not recognized as underlying cause for significant losses. While the total losses are significant, breeding for resistance has not been prioritized by most breeding programs. Contributing factors are complexity of the screening and consequent selection process, lack of resistance, insufficient information on which diseases to focus, since SBD often occur in combination and absence of reliable screening facilities. New breeding technologies like speed breeding, marker assistant recurrent selection, genomic selection, gene stacking, or gene editing would allow to make faster progress, provided SBD are prioritized by breeding programs. For this, more data from extensive yield loss trials in farmers' fields are needed. Every breeder knows that adding a complex trait like resistance to a SBD to the list of breeding priorities will slow down the progress for the other traits. Programs with limited resources located in areas where SBD is a serious constraint should utilize hubs to get reliable information about their lines. Giving the restructuring of the One CGIAR; SBD is a common problem and destructive threat for almost all 1CG mandate crops. For example, the root lesion nematodes (RLNs) are multi-host endoparasitic nematodes that have the actual potential to attack all the crops under the 1CG umbrella and obviously controlling RLN in one host crop will definitely lead to reduce its impact on other crops. Therefore, SBD should be given a common priority under the 1CG and not to be excluded.

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Identifying root rot (*Fusarium culmorum*) tolerant genotypes in wheat by using the RGB image-based vegetation indices

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Summary

Fusarium culmorum, also known as the crown rot fungus, causes significant damage to a wide variety of host plants worldwide. It is a difficult process to assess the susceptibility/tolerance of the genotypes. Digital image analyses are not only used to assess biomass and leaf area index, and for determining the chlorophyll content, radiation use efficiency, assessing drought, and in-season yield estimation but also used to determine and monitor mainly the foliar diseases in different crops. As the soil borne diseases affect the biomass and yield of the crop, RGB based vegetation indexes may be useful to assess the resistance/tolerance to soil borne diseases. The inoculated and non-inoculated trials were planted next to each other in Konya, Turkey in 2018-19 growing season. The photos were taken at different dates during the growing season and the yields were measured in both conditions. In average, the overall yield decrease was 7.9 % in the inoculated area compared to the non-inoculated area. The percent yield decreases were 12.8, 3.9, 7.4, 11.2, and 13.1 percent in resistance classes of 1, 2, 3, 4, and 5, respectively. The highest decrease was observed in the most susceptible genotypes with 13.1 %, the lowest decrease was in score 2 with 3.9 %. Correlations between RGB based image indexes and yield were similar to the Normalized Difference Vegetation Index (NDVI) reading, however the pattern in the Crop Senescence Index (CSI) was different from the others. Initial results indicates that RGB based vegetation indexes may have potential to assess the resistance/tolerance of the genotypes, however further detailed studies needed to assess the potential of it.

Introduction

Fusarium culmorum, also known as the crown rot fungus, is a soil-borne pathogen that causes significant damage to a wide variety of host plants (Nelson et al. 1993; El-Kazzaz et al. 2008). They can cause prominent disease symptoms in cereals such as crown rot, head blight, scab, and stalk rot. These symptoms have a negative impact on wheat production areas, resulting in yield losses worth billions of dollars (Windels 2000). *F. culmorum* is found in soil and plant matrices, including roots, aerial tissues, and organic substrates (Aoki et al. 2003). Cereal root rot symptoms include discoloration, necrosis, or rotting of the roots, sub crown internode, crown, and/or stem base (Stack 1982). Symptoms of severe root rot include stunting and wilting. RGB sensors are known to be useful for monitoring disease infection severity (Sugiura et al. 2016; Tetila et al. 2017; Vergara-Diaz et al. 2015; Feng et al. 2022). For plant phenotyping, RGB images are generally used to calculate NDVI, height, biomass, yield, etc. (Xie and Yang 2020). High-resolution RGB images have been used for the first time for wilt disease (*Fusarium oxysporum* f. sp. *raphani*) detection in radish (Dang et al. 2020). The objective of this study was to reveal the potential of RGB based imaging on the detection of tolerant wheat genotypes to *F. culmorum*.

Methods

Seventy-one advanced lines, with two checks, which are repeated 5 times in each replication were planted in Konya, Turkey to determine the yield loss under root rot inoculated conditions. The entries were planted as inoculated with *F. culmorum* and non-inoculated conditions; the trials were in the same field next to each other. The *F. culmorum* isolate was obtained from an infected wheat plant. The spore suspension was prepared by adding sterilized distilled water to each Petri dish containing *F. culmorum* culture. Wheat bran was inoculated with spore suspension. The fungus-colonized wheat bran was dried at room temperature and used for field experiments to inoculate the seeds of genotypes before planting.

Field experiments were conducted at Bahri Dağdaş International Agricultural Research Institute in Konya, Turkey in the 2018-2019 growing season. For each line, 140 g of seeds were planted in 6 m^2 rows and infested with an addition of 20 g of fungus-colonized wheat bran. The experimental trial design was Alpha Lattice with two replications in both conditions. Common agronomic practices for wheat were applied after planting: planting was done in October, 40/40 Kg/ha P_2O_5/N fertilizer was applied during the planting and additional 40 Kg/ha nitrogen was applied in spring during the tillering stage of the crop. Weed control was done by herbicides, and harvest was done in mid-July. Crown rot resistance-susceptibility responses of the entries were determined in a controlled environment, and they were scored from 1 to 5 on the following scale: 1 = Resistant (R), 2 = Moderately resistant (MR), 3 = Moderately susceptible (MS), 4 = Susceptible (S), and 5 = Highly Susceptible (HS). Digital photos and NDVI readings were taken every around 15 days starting from 06 March until 03 June during the growing season of the wheat. Photos were taken with a Sony a6000 camera (https://www.youtube.com/watch?v=O4oXwxVZLDM) using a focal length of 16 mm and keeping a constant distance between the camera and plot about 80-100 cm. The RGB digital image-based vegetation index, green area per meter square (GAm^2), Greener Green Area per meter square ($GGAm^2$), Early Vigor (EV), and Crop Senescence Index (CSI) were calculated using equations from BreedPix open-source software. NDVI measurements were made by handheld greenseeker "The Trimble GreenSeeker handheld crop sensor" (https://agriculture.trimble.com/product/greenseeker-handheld-crop-sensor/) at the same dates with photographs taken.

Spectral reflectance indices (SRIs) have been developed based on simple mathematical formulae, such as ratios or differences between the reflectance at given wavelengths. Simple ratio (SR=NIR/VIS) while Normalized Difference Vegetation Index (NDVI); NDVI= [(NIR-VIS)/(NIR+VIS)] = (R840-R670)/(R840+R670). These calculations were used to assess biomass and leaf area index. SRIs have been used for determining the chlorophyll content, radiation use efficiency, assessing drought, and in-season yield estimation.

ANOVA analysis was done for yield and correlations between RGB-based vegetation indexes and yield were calculated in Excel software.

Results

The number of entries in Resistance/Susceptible groups were 9, 20, 40, 6, and 6 from 1 to 5, respectively (Table 1). While the average yield was 4766.8 Kg/ha in non-inoculated area it was 4390.6 Kg/ha in inoculated areas, in average, the overall yield decrease was 7.9 % in the inoculated area compared to the non-inoculated area. The percent yield decreases were 12.8, 3.9, 7.4, 11.2, and 13.1 percent in resistance classes of 1, 2, 3, 4, and 5, respectively. The highest decrease was observed in the most susceptible genotypes with 13.1 %, the lowest decrease was in score 2 with 3.9%.

Table 1. The number of genotypes in each resistance/susceptible class, yield, and yield decrease of them under the non-inoculated and inoculated area

Resistance Class	The number of entries in the class	Average yield of the group in the non-inoculated area (Kg/ha)	Average yield of the group in the inoculated area (Kg/ha)	Yield decrease in inoculated area (%)	Yield decrease (Kg/ha)
1	9	4870.8	4248.3	12.8	622.6
2	20	4704.1	4522.6	3.9	181.5
3	40	4638.2	4295.5	7.4	342.7
4	6	5726.3	5086.8	11.2	639.4
5	6	4717.5	4101.3	13.1	616.3
Average		4766.8	4390.6	7.9	376.2

Correlations between digital images, NDVI, and yield under inoculated conditions are very low, non-significant, in the early growth stages of the plants (Figure 1), however it increases in the later crop growth stages. The correlations between yield and EV, GAm^2, GGAm^2, and NDVI in the first five measurements were negative, then they were high and positive in the last measurements. The correlations between CSI and yield under inoculated conditions followed a different pattern from the others; although there were not any statistically significant correlations in the first 3 measurements, it tended to be negative in next two measurements and reached the highest positive correlation in the measurement made on April 23 with 0.18. Then, the highest negative correlation was observed in the last measurement made on June 03.

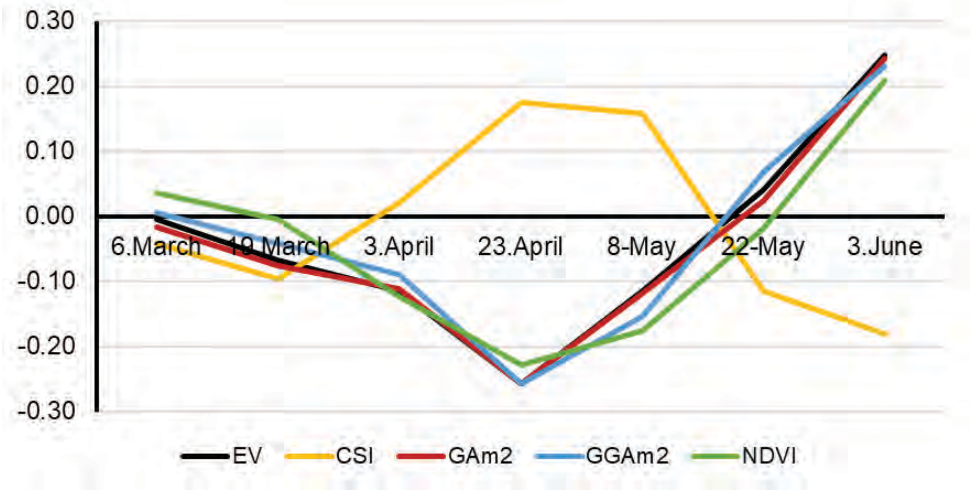

Figure 1. Correlations between yield and digital images under inoculated conditions. The X-axis represents the dates of measurements. The Y-axis represents the corresponding values for each vegetation index

Conclusion

RGB image-based vegetation indexes EV, GA, GGA and CSI could be used for estimation of potential grain yield at the early stage of crop growth, and RGB image-based vegetation indexes explained genetic variation in grain yield though correlations are very similar with NDVI. Initial results indicate that digital images and NDVI have the potential to assess the resistance/tolerance over the yield reduction of susceptible genotypes under inoculated conditions. However, more detailed studies are needed to confirm results and validation of the method's ability to identify clearly the resistant/tolerant genotypes. If a fast, cheap, repeatable, reliable and non-destructive screening method can be developed, higher number of genotypes can be screened in a shorter time to identify resistant/tolerant genotypes to RR/CR that could be used in breeding program. The RGB image-based vegetation indexes may have potential in that sense.

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Modified speed breeding scheme at ICARDA for the development of high yielding wheat varieties with resistance to major biotic and abiotic stresses targeting the CWANA and SSA regions

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Summary

Wheat production in the CWANA and SSA regions is significantly affected by abiotic (heat, drought) and biotic (rusts, septoria, nematodes, root rots, insects) stresses. The wheat breeding program at ICARDA develops high yielding wheat genotypes with resistances to these major stresses using classical and molecular approaches. A modified speed/shuttle breeding program involving two cycles of elite x elite crosses (simple crosses, F1top crosses while increasing F1s) and two cycles in the field (winter and summer seasons) at Merchouch station (Morocco) and Kulumsa station (Ethiopia) has been deployed. Genomic selection is applied for stage 1 trials at F6. Elite genotypes at F7 are evaluated across key locations: Wadmedani (Sudan) for heat tolerance, Merchouch (Morocco) for drought tolerance, Sids (Egypt) and Terbol (Lebanon) for yield potential and Kulumsa (Ethiopia) for resistance to diseases (rusts, Septoria). The whole process from crossing to elite germplasm distribution to NARS through international nurseries is completed in 4 years. Yield levels of the top yielding elite spring bread wheat genotypes ranged up to 6t/ha at Wadmedani station of Sudan under extreme heat stress, 7t/ha at Merchouch station of Morocco under terminal moisture stress (260-300 mm) and 11 t/ha at Sids station in Egypt under optimum conditions. Annually, ICARDA distributes more than 400 elite spring bread wheat genotypes to its partners through international nurseries. In the last 10 years alone, more than 60 bread wheat varieties of ICARDA origin have been released by National Agricultural Research System (NARS) in the CWANA and SSA regions.

Introduction

The Central and West Asia and North Africa (CWANA) and region is a vast geographic area extending east-west from the irrigated Indus Valley in Pakistan to the Atlas Mountains in Morocco; and from highland high rainfall areas of Ethiopia in the south to the very temperate and very dry northern Kazakhstan. As expected, this vast geographic area is characterized by large variation in agro-ecology, farming systems, moisture, temperature, soil types and cultural practices. Accordingly, it harbors all kinds of wheat of different growth habits: spring (61%), facultative (21%) and winter (18%). Wheat is the principal and staple food in most countries of the region, accounting for 45% of the region's per capita calorie intake with average wheat consumption of about 200 kg/capita/year, which is the highest in the world. According to FAO (2021), the CWANA region produces more than 120 million tons of wheat in a total area of 50 million hectares at a productivity level of 2.5 t/ha which is less than the worlds average (3.2 t/ha) (Tadesse et al 2015)

The low productivity of wheat in the region is due to abiotic stresses (drought, cold, heat, salinity) and biotic stresses (yellow rust, leaf rust, stem rust, nematodes, root rots, Russian Wheat Aphid, Barley Yellow Dwarf

Virus, Sunn pest, and Hessian Fly). Principally, drought and yellow rust are the most important wheat yield limiting factors. With the current climate change, it is anticipated that new pests and diseases will emerge as already exemplified in the recent epidemics of yellow rust across the CWANA region and Ug99 epidemic in East African countries. The effect of climate change is also evident on the quality of wheat as increased heat results in shriveled wheat grains (Braun et al 2010; Tadesse et al 2020).

ICARDA, located in the heart of the Fertile Crescent where wheat originated, started wheat improvement program since its inception to develop high yielding and widely adapted wheat varieties with resistance to the major biotic and abiotic stresses and better end use quality targeting the Central and West Asia and North Africa (CWANA) regions and beyond.

Development of wheat varieties: Breeding approaches and strategies

Wheat breeders have been trying to adopt breeding strategies such as shuttle breeding, doubled haploids, and single seed descent (SSD) schemes to rapidly advance segregating and there by shorten the breeding cycle and increase the rate of genetic gain. Recently, speed breeding scheme is being highly promoted for accelerated breeding and higher rate of genetic gain. Speed breeding' technology is the production of up to six generations of wheat per year involving extended duration of light and early harvesting of wheat under glasshouse conditions. This enables effectively to shorten the breeding cycle through acceleration of generation advancement. However, it might be limited in its scope to screen the population for different biotic and abiotic stresses in addition to the difficulty of handling of large number of populations required at F_2 stage. The wheat breeding program at ICARDA uses classical and molecular approaches to develop well adapted high yielding germplasm with resistance to the major biotic and abiotic stresses. The program has adopted a modified speed breeding program where the crosses and F1 production are carried out in the plastic houses followed by summer and winter shuttle of the F2, F3 and F4 head rows at Merchuch (Morocco) and Kulumsa/Melkaworer (Ethiopia). The stage 1 (preliminary yield) and stage 2 (advanced yield) trials are carried out during the main seasons at key locations: Merchouch and Settat (Morocco) for drought tolerance, Terbol (Lebanon) for cold tolerance and adaptation, Sids (Egypt) for yield potential, Wadmedani (Sudan) for heat tolerance and Kulumsa (Ethiopia) for resistance to diseases (yellow rust, stem rust, septoria etc). A total of 3000 and 600 wheat genotypes are tested in stage 1 (PYT) and stage 2 (9AYT) trials, respectively across the key locations: Wadmedani (Sudan) for heat tolerance; Merchouch and Sid Al Aydi (Morocco) for drought tolerance and resistance to nematodes and Hessian fly; Sids (Egypt) for yield potential; Terbol (Lebanon) for cold tolerance, adaptation and resistance to sun pest; and Kulumsa (Ethiopia) for resistance to diseases (rusts, septoria). The whole process from crossing to elite germplasm distribution to NARS through international nurseries is completed in 4 years. It should be noted that in this modified speed breeding scheme, we are using elite x elite crosses, selection of spikes for head-rows in the F3 and it requires rouging at the head row, PYT and AYT stages. This method is rapid, efficient and enables to attain fast genetic gain. The protocol is published in the wheat breeding handbook (Tadesse et al 2019).

Yield levels of the top yielding elite spring bread wheat genotypes ranged up to 6t/ha at Wadmedani station of Sudan under extreme heat stress, 7t/ha at Merchouch station of Morocco under terminal moisture stress (260 -300 mm) and 11 t/ha at Sids station in Egypt under optimum conditions.

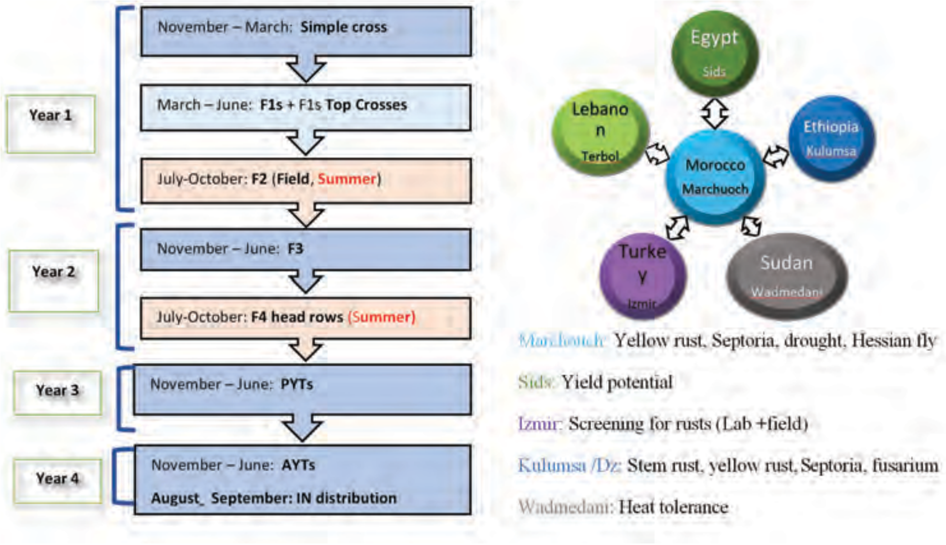

Figure 1. Wheat breeding schemes and key locations (research hubs) at ICARDA.

Germplasm distribution and variety release

Annually, ICARDA distributes more than 400 elite spring bread wheat genotypes to its partners through international nurseries. National programs carry out national or regional variety trials for 2-3 years followed by a 1-year variety verification/registration trials of 2-3 candidate varieties along with their national and local check varieties (Fig 2).

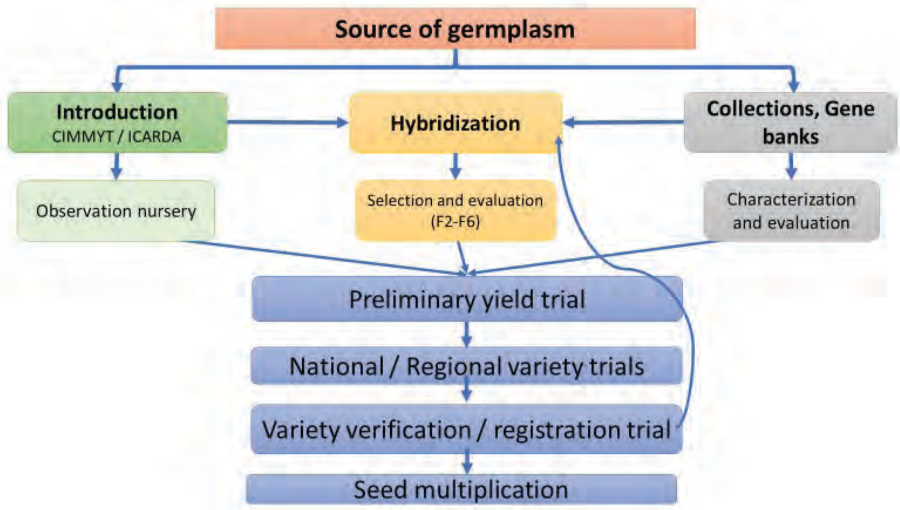

Figure 2. Variety development, evaluation, and release scheme in the natio.nal programs (Tadesse et al. 2022).

Using such process, in the last 10 years alone, more than 65 bread wheat varieties of ICARDA origin have been released by National Agricultural Research System (NARS) in the CWANA and SSA regions. Moving forward, variety release by its own will not bring impact unless it is supported by a strong seed multiplication and diffusion scheme. To this end, it is important to strengthen the national breeding programs for continuous variety development and timely supply of breeder seeds for newly released varieties; build the capacity of the national seed enterprises for production of certified seed; formulate conducive policies along the wheat value chain and establish strong public-private partnership.

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International winter/facultative wheat elite germplasm: screening for resistance to soil borne pathogens in CIMMYT-Türkiye

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Summary

The International Winter Wheat Improvement Program (www.iwwip.org) develops germplasm adapted to Central and West Asia combining broad adaptation with resistance to prevailing abiotic and biotic stresses. IWWIP also plays a key role in facilitating global germplasm exchange among the winter wheat breeding programs. Germplasm developed by IWWIP as well as the material received from cooperators is distributed as Observation Nurseries and Yield Trials to more than 100 cooperators in 50 countries.

In this study, 982 wheat lines from Candidate International Winter Wheat Yield Trial Nurseries Irrigated & Semi-arid (CAND-IWWYT-IRR&SA), representing a broad geographical spectrum of breeding lines and varieties from Türkiye, Europe, USA, CIMMYT-Mexico, Central Asia, Iran, and the International Winter Wheat Improvement Program (IWWIP), have been screened against Cereal Cyst Nematodes (*Heterodera filipjevi)* and Crown Rot (*Fusarium culmorum*), under controlled conditions (growth room and greenhouse) by the Soil Borne Pathogen Program (SBP) team in Türkiye. One hundred thirty and 136 lines were scored resistant and moderately resistant to *H. filipjevi*, representing 13.2% and 13.8% of the screened germplasm, respectively. Out of the 446 wheat germplasm, 4 (0.9%) ranked as resistant and 18 (4%) moderately resistant (MR) to a local isolate of *Fusarium culmorum* under controlled greenhouse and growth room conditions. The best lines in terms of resistance for CCN-*H. filipjevi* and Crown rot-*F. culmorum* have been distributed to cooperators in 50 countries.

Introduction

Wheat is grown on 20% of the cultivated land area of the world and is the main food resource for 40% of the world's population (Braun et al. 2010). Among the biotic stress factors, *Fusarium species* causing foot, crown, and root rots occur in winter cereals worldwide virtually wherever cereal-based farming systems predominate (Burgess et al., 2001). In the drylands of Central and West Asia and North Africa (CWANA) soil-borne diseases (like crown rot) and various nematode species reduce the grain yield by up to 40%. These diseases and pests benefit from higher temperatures when they become more active (Colagiero and Ciancio 2011). The International Winter Wheat Improvement Program (IWWIP) is a cooperative breeding program of the Turkish Ministry of Food, Agriculture, and Livestock; the International Maize and Wheat Improvement Center (CIMMYT); and the International Center for Agricultural Research in the Dry Areas (ICARDA). IWWIP aims to develop broadly adapted lines for irrigated and semi-arid areas of CWANA. More than 120 winter wheat varieties originating from IWWIP germplasm have been released in the target region and are grown on more than 2.5 mln ha. IWWIP-derived varieties are characterized by higher yield potential, drought tolerance, and disease resistance. Substantial additional income to farmers and the environment comes from reduced fungicide application on disease-resistant varieties (Morgounov et al. 2019).

A program for screening, validating, and disseminating SBPs resistant/tolerant germplasm has been established linking CIMMYT Mexico and IWWIP in Türkiye. One of the major components of the SBPs program work at CIMMYT-Türkiye involves screening of IWWIP developed elite lines and/or germplasm originating from different countries. The main objective is to work with plant pathologists to transfer SBPs resistance into adapted wheat backgrounds. For this purpose, IWWIP annually provides around 500 winter wheat lines comprised of facultative winter wheat observation nurseries (FAWWON) to screen winter wheat germplasm against SBPs.

Methods

Candidate lines from the 15Cand.IWWYT-IRR&SA to the 21Cand. IWWYT-IRR&SA nurseries, distributed between 2015 to 2021. Nine hundred eighty-two lines from 18 countries were evaluated for resistance to *H. filipjevi* and 446 lines for resistance to *F. culmorum*.

Screening for Nematodes: Screening tests were carried out using a population of *H. Filipjevi* collected from the naturally infested field in Kırsehir, Türkiye (39º 39′ 709″ N, 32º 37′ 14″ E) molecularly identified according to the Peng et al. 2013.

Growth Room Screening: Single pregerminated wheat seed was planted in standard small tubes (16 cm in height × 2.5 cm in diameter) containing a sterilized mixture of sand, field soil, and organic matter (70:29:1 v/v/v). The field soil and sand were sieved and sterilized at 110 °C for 2 h for 2 successive days, and the organic matter was kept at 70 °C for 5 h. At the sowing date freshly hatched 300 juvenile/ml were inoculated on the soil around the stem base. The plants were grown in a growth chamber with a 16-h artificial photoperiod and maintained at a temperature of 22 ± 3 °C with 70% relative humidity (Erginbas et al. 2018).

Screening for Fusarium: A local *Fusarium species* isolated from a naturally infested field in Kırsehir, Türkiye (39º 39′ 709″ N, 32º 37′ 14″ E), molecularly identified as *F. culmorum* according to Nicholson et al. (1998) was used in all tests (Erginbas et al. 2018).

Greenhouse Screening: A single wheat seed was sown in each plastic tube (Stuewe and Sons, Corvallis, OR) (21 cm height x 3.8 cm diam.) filled with the same potting mixture as mentioned above and was inoculated with 0.25 g of wheat bran *F. culmorum* consisted of about 5x10^5 spore per ml. Wheat bran was soaked in 1 ml water to estimate relative spore concentration by using a hemocytometer. The plants were left to grow under the greenhouse from October to June (winter wheat growing season) and harvested at maturity as lined with natural field conditions (Erginbas et al. 2018).

Experimental Design: Each treatment was replicated 3 times and tubes were placed in a completely randomized block design (CRBD).

Harvest: *Nematode-*; The plants were harvested 9 weeks after juvenile inoculation. Soil from each tube was collected in a 2-L pot filled with water for cyst extraction, while roots were washed on nested sieves with 850 µm and 250 µm mesh sizes to free cysts from the root system. Cysts from both root and soil extractions were collected on the 250 µm sieve and counted under a stereomicroscope. *Crown Rot-*; The germplasma were ranked according to the browning/rotting severity on the crown and were compared to the controls used in this study (Erginbas et al. 2013a).

Assessment: *Nematode-*; The grouping was performed based on cyst number per plant compared to the cyst numbers on the known checks. The following ranking was followed; 1= resistant (R), 2= moderately resistant (MR), 3= moderately susceptible (MS), 4= susceptible (S) and 5= highly susceptible (HS) (Dababat et al. 2019). *Crown Rot-*; Infested plants were harvested and assessed based on the browning/rotting percentage on the crown which describes the stem (1 cm above soil level) according to the modified 1-5 scale: 1= 1-9% Resistant (R), 2= 10- 29% Moderately Resistant (MR), 3= 30-69% Moderately Susceptible (MS), 4= 70-89% Susceptible (S), 5= 90-100% Highly Susceptible (HS) (Erginbas et al. 2013a).

Results

In this report, all SBPs screening data of the CAND-IWWYT-IRR&SA generated between 2015-2021 provided by the SBP team is collated and presented. The cyst number ranged from 1 to 41. The tested lines showed a disease severity ranging between 1% and 89%. The lines were categorized into 5 groups based on their reaction ranging from Resistant (R) to Highly Susceptible (HS). The screening of 982 winter wheat germplasm resulted in identifying 13.2% as resistant, and 13.8% as moderately resistant to *H. filipjevi* in the growth room (Table 1). The screening of 446 winter wheat revealed that 0.9% of germplasm were resistant, and 4% were moderately resistant to *F. culmorum* in the greenhouse (Table 2). A total of 262 and 22 germplasm selected for CCN-*H. filipjevi* and CR-*F. culmorum* were distributed to cooperators (Table 3).

Table 1. Percentage of resistant and moderately resistant lines to Cereal Cyst Nematodes, *Heterodera filipjevi* in Candidates International Winter Wheat Yield Trials Nurseries

Nursery Name	Total tested	Group 1- Resistant				Group 2- Moderately Resistance			
		IRR/SA Germplasm	%	Globally distributed germplasm	%	IRR/SA Germplasm	%	Globally distributed germplasm	%
21CIWWYT-IRR&SA	137	8	6	3	2	12	9	3	2
20CIWWYT-IRR&SA	162	13	8	8	5	13	8	4	2
19CIWWYT-IRR&SA	157	18	11	11	7	29	18	20	13
18CIWWYT-IRR&SA	150	26	17	13	9	13	9	5	3
17CIWWYT-IRR&SA	147	22	15	11	7	43	29	21	14
16CIWWYT-IRR&SA	98	23	23	13	13	8	8	4	4
15CIWWYT-IRR&SA	131	20	15	11	8	18	14	10	8
TOTAL	**982**	**130**	**13.2**	**70**	**7.1**	**136**	**13.8**	**67**	**6.8**

Table 2. Percentage of resistant and moderately resistant lines to Crown Rot, *Fusarium culmorum* in the Candidates International Winter Wheat Yield Trials Nurseries

Nursery Name	Total tested	Group 1- Resistant in GR&GH				Group 2- Moderately Resistance in GR&GH			
		IRR/SA Germplasm	%	Globally distributed germplasm	%	IRR/SA Germplasm	%	Globally distributed germplasm	%
21CIWWYT-IRR&SA	138	1	0.7	1	0.7	5	3.6	2	1.4
19CIWWYT-IRR&SA	158	1	0.6	1	0.6	6	3.8	4	2.5
18CIWWYT-IRR&SA	150	2	1.3	2	1.3	7	4.7	3	2.0
TOTAL	**446**	**4**	0.9	**4**	0.9	**18**	4.0	**9**	2.0

Table 3. The number of screened and globally distributed germplasms selected resistance against to the Cereal Cyst Nematode *Heterodera filipjevi* and Crown Rot *Fusarium culmorum*

Country	Total numbers of entries	CCN-number of resistant (group 1&2) entries	%		Total numbers of entries	CR-GH number of resistant (group 1&2) entries	%
Azerbaijan	3	0	0		3	0	0
Belarus	1	0	0		1	0	0
Bulgaria	4	0	0		-	-	-
Switzerland	5	0	0		5	0	0
Croatia	2	1	50		-	-	-
Germany	5	2	40		5	0	0
Georgia	1	1	100		1	0	0
Hungary	11	5	45		4	0	0
Iran	25	4			13	1	7.7
Kazakhstan	14	4	29		6	0	0
Morocco	3	1	33		3	0	0
Romania	20	2	10		11	1	9.1
Tajikistan	3	0	0		3	0	0
Russia	26	3	12		17	0	0
Ukraine	5	0	0		5	0	0
USA	140	45	32		70	4	5.7
Turkiye	58	13	22		14	0	0
IWWIP-USA	75	23	31		39	4	10.3
IWWIP-Mexico	71	23	32		9	0	0
IWWIP	510	139	27		237	12	5.1
TOTAL	**982**	**266**	**27**		**446**	**22**	**4.9**

Discussion

Using resistant lines with high yield is the most effective and economical way to control SBPs, especially in drought-affected areas where cereals are cultivated and monoculture cropping systems exist (Erginbas-Orakci et al. 2013b). The performances of wheat lines tested in field trials in Türkiye and CWANA have indicated that some of them combine resistance to CCN and Crown Rot with competitive grain yield and resistance to yellow and stem rust. These lines have been extensively used as parental lines the most competitive lines may be released cultivars. Particular attention must be paid to managing SBPs through the introduction of resistance genes in widely grown cultivars, since an evaluation is needed regarding the persistence of the genetic pool introduced, on a scale of decades or years. Breeding for SBPs is extremely still a bottleneck and should be given more attention. More information about breeding challenges can be found in Braun's paper in this book.

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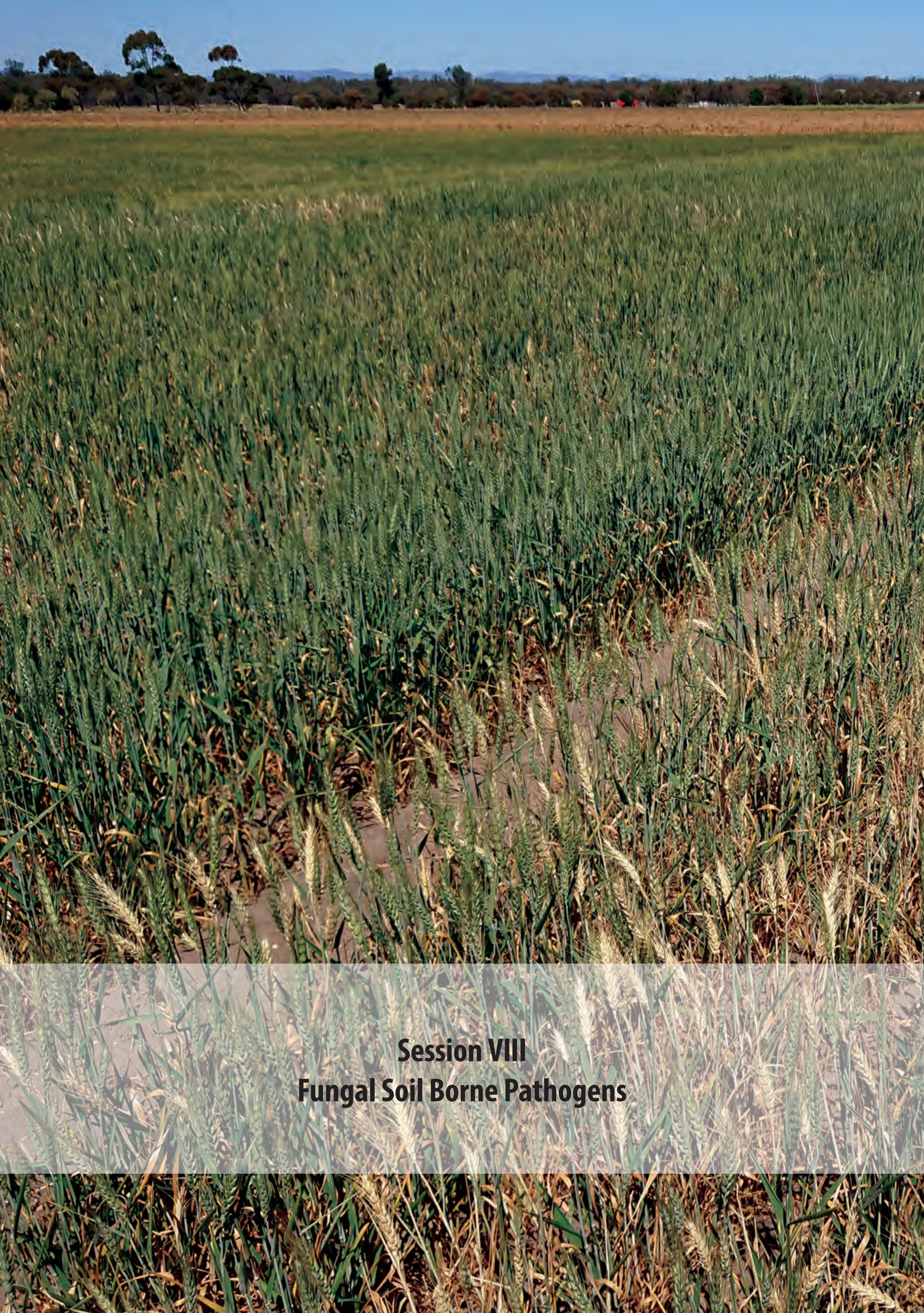

Session VIII

Fungal Soil Borne Pathogens

The wheat microbiome and soil health-making the connections

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Introduction

Soilborne plant pathogens do not exist in a biological vacuum in the soil. The soil is one of the most complex (and least understood) ecosystems in the world. Besides large populations of bacteria, archaea, algae, and fungi, there is a complex of microfauna (protists, ciliates, paramecia, rotifers, amoeba, flagellates, nematodes) and soil invertebrates (collembola, insects, mites, millipedes, earthworms and other annelids, ants, and termites). Thus, a soilborne pathogen propagule or hyphae in the soil may encounter all of these soil community members, but how do they affect the success of the pathogen? Except for research on biological control and suppressive soils, which focus on one or a few species of antagonist, the answer to this question is mostly unknown. As plant pathologists, we tend to focus on the plant pathogen and often do not consider the rest of the soil community. Over the last decade, the topic of soil health has become one of the hottest topics in soil science and soil microbiology. In the last 5 years, over 21,000 papers have been published on soil health. But what does soil health mean, and how can it be measured? This is a controversial topic still not resolved. Until the development of high throughput DNA techniques, soil microbiologists measured soil quality indicators such as organic matter, aggregate stability, porosity, C, N, available permanganate oxidizable C, or readily mineralizable C through short term microbial activity measured by CO^2 release. A number of enzyme assays quantify key components of the C, N, and P cycling, such as N-acetyl-β-D-glucosaminidase, β-glucosidase and acid/alkaline phosphatase. But none of these methods can distinguish individual taxa. Phospholipid fatty acid profile (PLFA) fatty acid methyl ester profile (FAME) have been used the last 30 years and are based on quantifying specific fatty acid profiles in the membranes of bacteria and fungi.

However, these techniques only provide broad resolution snapshots – i.e. gram positive or gram negative bacteria, fungi, AMF fungi, etc. The development of next-generation sequencing methods, first pyrosequencing and now Illumina MiSeq, PacBio, nanopore, now provides the ability to identify thousands of taxa of fungi and bacteria from a single soil or plant sample. The details can be found in the reference papers. To summarize, total DNA can be isolated from soil, rhizosphere and roots using extraction kits, and amplicon sequencing can be used with ITS or 16S primers, which target a taxonomically informative section of the ribosome. This PCR reaction specifically amplifies fungi or bacteria sequences, and thousands of short sequences can be generated from a single sample. Using standard bioinformatics pipelines, these sequences can be identified to various taxonomic levels using sequence databases, usually to the family or genus level. In this paper, we will summarize some of our findings on dryland wheat microbiomes in the Inland Pacific Northwest of the US over the last 15 years.

Who is present: the wheat core microbiome

Schlatter et. al. (2020a) examined the bulk soil collected from four precipitation/cropping system zones over two years in eastern Washington, both cropped and adjacent non-cropped land. They grew wheat plants in the greenhouse and extracted bulk soil and rhizosphere DNA. In the bulk soil, location was a major driver of diversity of bacteria. But in the rhizosphere, location had less of an effect, and the plant selected a narrower set of core taxa. The core set of bacteria found in >95% of rhizosphere or bulk soil samples were including members of *Bradyrhizobium,* Sphingomonadaceae, *Massilia, Variovorax,* Oxalobacteraceae, and Caulobacteraceae. There was a stronger rhizosphere effect with bacteria than fungi and fewer core fungal taxa. Core fungal taxa in the rhizosphere included *Nectriaceae, Ulocladium, Alternaria, Mortierella,* and *Microdochium*. Co-occurrence networks identified *Sphingomonas, Massilia, Microdochium* and *Knufia* as key hub tax. Fig. 1 shows the phylogenetic placement of the core bacteria taxa in the rhizosphere- actinobacteria, betaproteobacteria (primarily Oxalobacteraceae) and alphaproteobacterial (especially *Sphingomonas*). This work shows that the wheat rhizosphere can select a core microbiome from the surrounding soil.

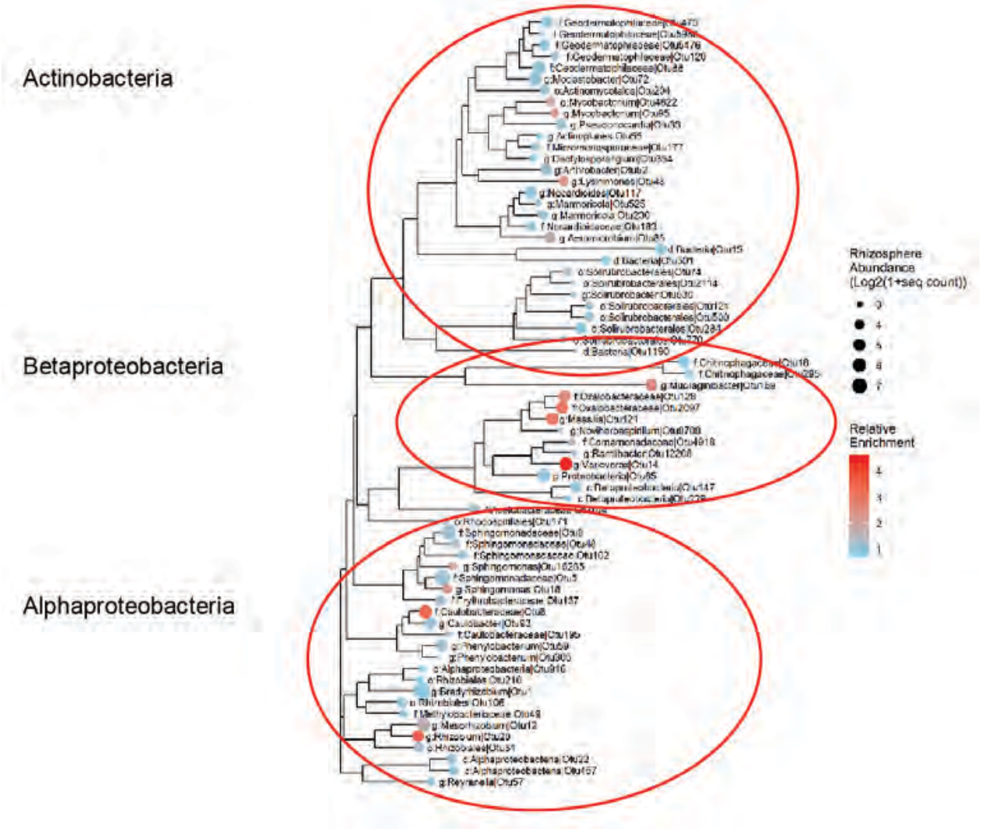

Figure 1.

How do previous rotation crops affect the wheat microbiome?

There is increasing evidence that the previous rotation crop can affect the following wheat microbiome. We completed a 6-year rotation study with spring wheat following winter canola, winter wheat, winter triticale, spring barley or continuous spring wheat. This research was initiated to explain why there were yield decreases in wheat after winter canola, contrary to the dogma that rotation crops increase yield of the following wheat crop (Schillinger and Paulitz 2018). Fig. 2 shows that winter canola significantly changed the microbiome of the following wheat crop. In addition, canola suppresses AMF (arbuscular mycorrhizal fungi) in the following wheat crop, because canola is non-mycorrhizal.

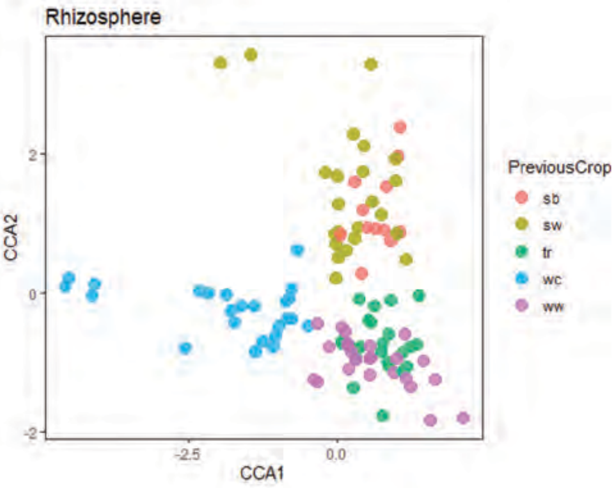

Figure 2.

Where are the communities? Soil depth is a major driver

Besides knowing what taxa make up the community, it is important to know the spatial variability- across landscapes, geographic areas, and within a field. One of the most important factors structuring microbial communities is soil depth. Schlatter et al. 2020b sampled at the Cook Agronomy Farm across a topographically variable landscape at 0 cm, 10 cm, 25 cm, 50 cm, 75 cm, and 100 cm, and used amplicon sequencing to define the bacterial and fungal communities. Because of direct-seeding and placement of ammonia fertilizer in the seed zone for over 20 years, there was an acidified layer at 10 cm that contained less diversity and richness and a unique community composed of Rhodospirellaceae and Koribacteraciae. The shallower upper layer was dominated by copiotrophs and root associated taxa in the shallow layer- *Flavobacterium*, Oxalobacteria, *Caulobacteria,* Sphingobacteria. The lower depths have decreasing C and increasing pH and were dominated by oligotrophs- actinobacteria (Gaiellaceae) and *Nitrospira*. Fig. 3 shows an NMDS plot of the communities in the different depths, each with a distinct community. In the lower depths, a greater portion of taxa were not able to be identified, indicating novel taxa that have not been described.

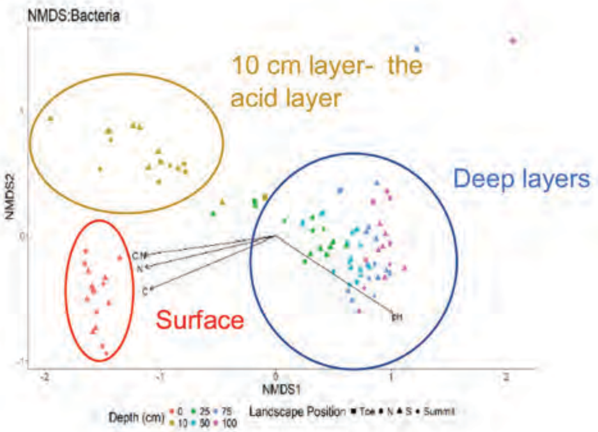

Figure 3.

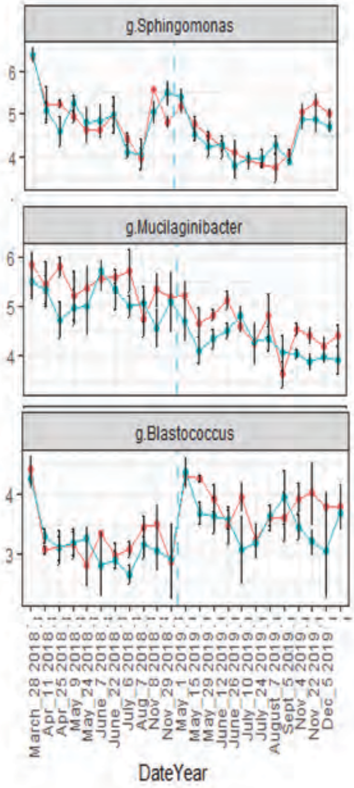

Figure 4.

When the temporal dynamics of communities in the bulk soil

Due to cost limitations, most studies tend to just look at a small number of sampling times. To shed light on temporal dynamics of soil microbiomes, we conducted a three-year experiment of sampling every two weeks during the growing season at the Cook Farm LTAR (Long Term Agricultural Research Site) (Fig. 4). Temporal dynamics varied among taxa. For example, *Sphingomonas* abundance is highest in the early spring, when moisture is highest, and then declines over the summer as the soil dries. This location in Eastern Washington State has a Mediterranean climate, with most precipitation as rain in the late fall, snow during the winter, and rain in the spring. During the summer months and main growing season (May, June, July, and Aug), rainfall is scarce, and plants rely on stored soil moisture in the deep loess soil profiles. The differences between the two years for *Mucilaginibacter* and *Blastococcus* may reflect the previous rotation crop (wheat in 2017 and chickpeas in 2018). *Muciliganibacter* is a well-described rhizosphere bacterium of wheat.

How do the microbial communities benefit wheat?

Out of the thousands of bacterial and fungal species, can we identify those that increase or decrease yield? Let the field tell us. At the Cook Agronomy Long Term Agricultural Research (LTAR) site, we have over 25 years of data from GPS sites around the approximately 30 ha of each farm. Cook East is an aspirational, directed seeded farm for the last 25 years (Figs. 5 and 6). Cook West is a business as usual, with a reduced tillage chisel plowed practice. Fig. 5 shows the organic matter levels of the two farms. Twenty-five years of no-till (direct seed) has resulted in about a 1% increase in OM. But pH is lower in the direct seed farm (Cook East) because of nitrification from applying ammonium to the 10 cm layer and not mixing the acidified soil into the soil profile. Do these tillage systems affect the microbial communities? We sampled both farms at sites indicated by the crosses, at 0-10 and 10-20 cm and defined both the bacterial and fungal communities at each location.

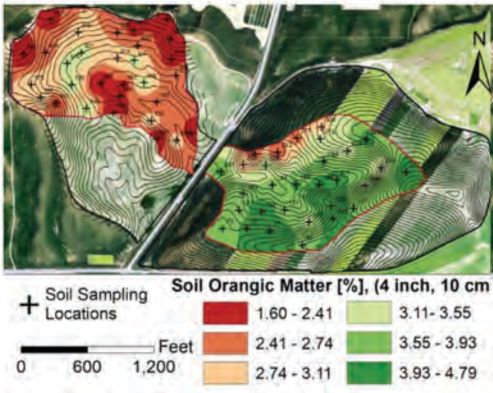

Figure 5.

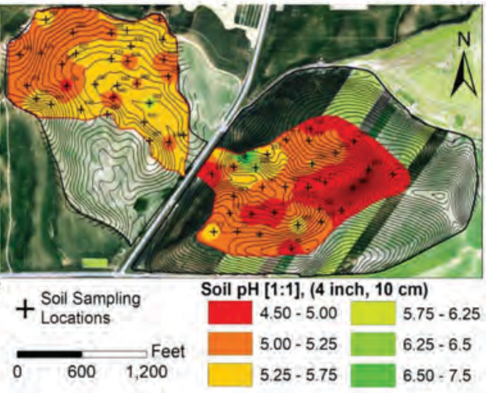

Figure 6.

We also correlated the abundance of all the individual taxa with soil chemical and edaphic factors and the relative yield. Fig. 7 shows the NMDS ordination of the bacterial communities. In the upper layer (red), we could separate out the two cropping systems. But at the deeper levels, cropping systems had less of an effect on community composition.

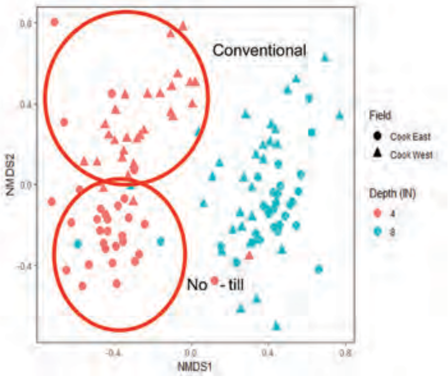

Figure 7.

What taxa are most predictive of yield? We had hypothesized that PGPR such as *Pseudomonas* or *Flavobacterium* would be positively correlated with yields, but in most cases there were negative relationships. In contrast, a number of fungal families such as Clavicepitaceae and Sordariaceae showed strong positive correlations (Fig. 8). These fungi are probably involved in residue degradation and carbon cycling. Phaeosphaeriaceae, a family which contains plant pathogens, showed negative correlations, along with Glomeraceae, arbuscular-mycorrhizal fungi. This was surprising, because AMF fungi are usually beneficial to plants. One possibility is that AMF fungi in wheat are parasitic, because with high P levels already in the soil, they take carbon but do not provide a benefit. The other possibility is that low yielding sites are low yielding because of soil and edaphic factors- i.e. are poor soils. In these situations, the stressed plants may recruit more AMF fungi and beneficial bacteria, thus they show a negative correlation. But the real test to separate out correlation from causation, is to isolate specific bacteria and fungi from the soil, and test them individually in greenhouse assays to see if they increase plant growth, and protect against drought stress or root diseases. We have started a collection of bacteria from wheat, but have a more extensive collection (3000+) isolates from camelina, a biofuels brassica. Wheat and camelina share many rhizosphere bacteria, so we can also test these on wheat.

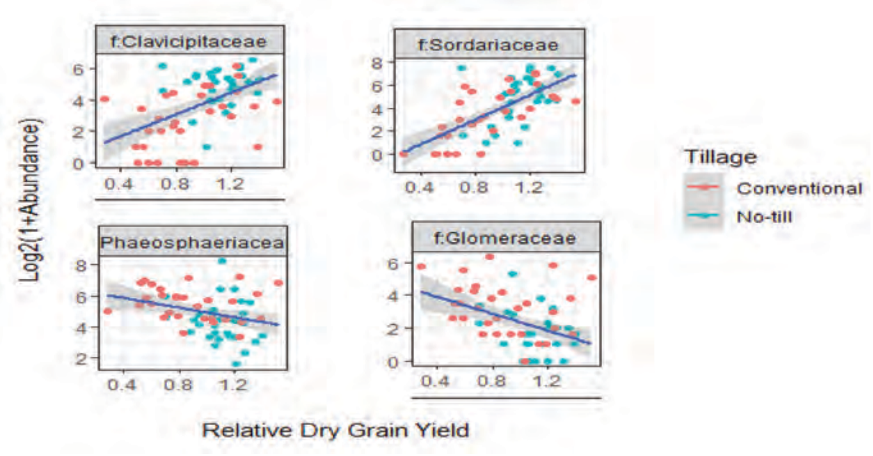

Figure 8.

Conclusions

Understanding the microbiome of wheat is critical for optimizing wheat health, because of the role individual communities may play in suppression of soilborne pathogens. In the past, we focused on just a narrow subset of the microbes. But the entire community may act to provide resiliency to wheat by protecting against abiotic stress such as drought, by increasing the efficiency of uptake of limiting nutrients, especially P which is not renewable.

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The impact of *Fusarium* crown rot of wheat in Australia

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Summary

During the past 20 to 30 years, *Fusarium* crown rot (FCR, caused by *Fusarium pseudograminearum* and/or *F. culmorum*) has become one of the most important diseases of wheat crops in Australia. As a stubble borne disease of cereals and grasses its prevalence has increased with the widespread adoption of stubble retention cropping practices and intensification of cereals in the cropping system. A recent study of the economic impact of FCR in Australia, based on inoculum levels in 5.993 fields (2015 to 2017) determined using qPCR molecular analysis (PREDICTA®B), estimated average annual losses of 6% (A$404 million) with losses of 8-10% possible across the regions. These losses were estimated using the negative relationship between pre-sowing inoculum density in the field and grain yield in seasons with hot and/or dry conditions during grain filling; with yield loss often not measured in seasons with a wetter and cooler seasonal finish. Australian growers can use PREDICTA®B testing commercially to identify fields at risk of FCR infection to plan rotations to minimise loss from this disease.

Introduction

Fusarium crown rot (FCR), caused by the fungal pathogens *Fusarium pseudograminearum* and/or *F. culmorum*, is an important disease of all winter cereal crop species in Australia. This disease has increased in importance with the widespread adoption of stubble retention practices and intensification of cereals within cropping systems (Burgess et al. 2001). FCR is more severe in seasons with low spring rainfall (i.e., a dry finish to the season) (Hollaway and Exell 2010). Management is difficult due to limited tolerance in commercial wheat cultivars and a lack of registered effective chemical options. A pre-plant qPCR soil/stubble test (PREDICTA®B) provides growers with a valuable tool to identify fields at risk of loss due to FCR and implement available integrated management strategies (Hollaway et al. 2013).

This paper reports on a major study undertaken to estimate losses to the Australian wheat industry due to FCR. Information on the distribution of the causal agents of FCR, based on 5.993 qPCR test results (2015-2017) from commercial fields, were combined with relationships between inoculum level and yield loss in a range of seasons and production regions to determine yield and economic losses from FCR. This is the largest study of impacts of FCR of its type internationally and relied on extensive research conducted nationally during the previous decade into the impact and management of FCR in wheat to estimate disease impacts nationally.

Methods

Distribution of Fusarium crown rot in Australia

Data on the distribution and level of FCR across Australia over the three-year period 2015-2017 were collected from 5.993 soil samples with added stubble when present taken from fields in wheat growing areas and tested by the commercial qPCR testing service, PREDICTA®B. The presence of FCR in a sample was based on a positive detection from one or more of three tests (tests 1 and 2 for *F. pseudograminearum* and a third test for *F. culmorum/F. graminearum*) conducted on each sample.

For the purpose of this study, as shown in Figure 1, Australia's grain production area was divided into three regions: North (Queensland and New South Wales), South (Victoria, South Australia and Tasmania) and West (Western Australia) as defined by the Grains Research and Development Corporation (GRDC). These regions were further broken down to agro-ecological zones (Figure 1) so that calculations of economic impacts could be made at a level that allowed for differences in soil types, yield potential and varieties grown within different parts of each region. The zone-level impacts were aggregated to Regions and then to national estimates of the overall impacts.

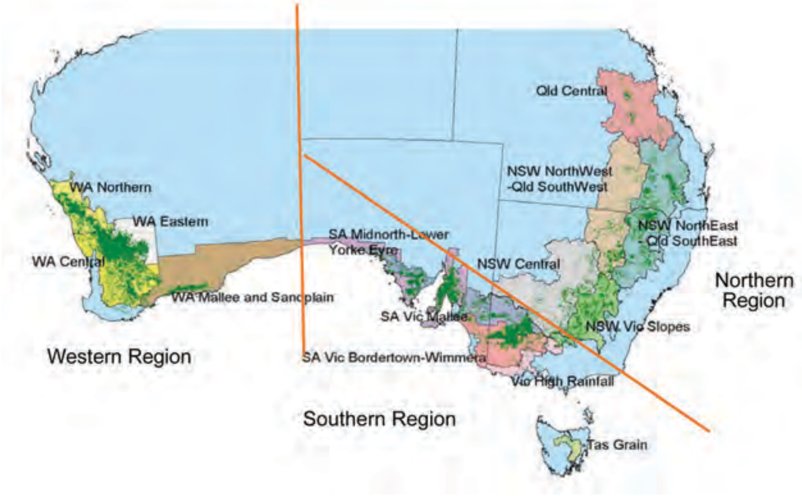

Figure 1. Three broad-acre cropping regions (Western, Southern and Northern Region) and agro-ecological zones in Australia.

Impacts of Fusarium crown rot in wheat

Information on FCR distribution and levels within each region was combined with estimates of yield loss based on inoculum level, frequency of yield loss based on regional climatic condtions, relative tolerance of varieties grown within a region, regional grain yield and price to determine the economic impacts of FCR within a region and nationally.

Wheat production data

Wheat production data were sourced from the Australian Bureau of Statistics averaged over five years 2013–2017. The average area of wheat was 12.2 million hectares, with an average yield of 1.99 t/ha, giving an average national production of 24.3 million tonnes. At an average unit value of wheat grain over the five years of $A282 per tonne, the total gross value of wheat production averaged $6.87 billion per year.

Estimates of yield loss from Fusarium crown rot

Yield losses in varieties with different FCR resistance ratings, in different zones within each region and in different seasons were made by researchers from each region for each of four pre-sowing FCR risk categories (not detected, low, medium and high). These loss estimates were informed by multiple field experiments conducted in a range of seasons and environments, including those reported by Hollaway et al. (2013). Within these experiments, different pre-plant inoculum levels were established in the field and quantified pre-sowing using qPCR (PREDICTA®B). The field experiments were then planted to cereals and subsequent disease development and grain yield measured. This allowed yield loss estimates for different pre-plant inoculum levels in different environments and different seasons to be calculated across Australia.

Varietal reaction to infection by Fusarium crown rot

Wheat varieties, rated for their resistance to FCR infection, were grouped into three broad categories: "Very susceptible" (VS/S-VS); "Susceptible" (S/MS-S); and "Moderately susceptible" (MS). The highest level of resistance currently available in wheat varieties is MS. The distribution of varieties planted by growers within these different FCR resistance groups within each Region was determined from grain deliveries over this period (Table 1).

Table 1. Percentage of wheat crops in each of three regions and nationally with different ratings towards Fusarium crown rot based on actual production data

Resistance rating group to Fusarium crown rot	Proportion (%) of wheat area sown in region, 2016			
	Northern	Southern	Western	Australia
Very susceptible	3	1	0	1
Susceptible	53	90	98	82
Moderately susceptible	44	9	2	17

Seasonal conditions

Seasons were characterised as having been conducive, intermediate and non-conducive to FCR in relation to long-term averages of end of season rainfall. The proportion of each of these seasons within each zone was estimated by local researchers. As an example, the frequency of occurrence of conducive, intermediate and non-conducive varied from 0 to 70%, 10 to 30% and 10 to 90%, respectively in the different zones that make up the southern region.

Results

Distribution of Fusarium crown rot in Australia

Fusarium crown rot was widely distributed and present in approximately half of all samples tested nationally (Table 2). The incidence of detection ranged from 39% in the Western region to 64% in the Southern region (Table 2). Nationally, high levels of inoculum were detected in 17% of samples tested, while in the Southern region 26% of samples had high FCR levels at sowing from 2015–2017.

Table 2. Number of samples tested, incidence and level of Fusarium crown rot in samples collected in the three grain growing regions of Australia as tested using qPCR (PREDICTA®B) during 2015-2017

Region	Number	Incidence (%)	Frequency (%) in each risk category		
			Low	Medium	High
Northern	2.491	42	24	5	14
Southern	1.502	64	28	10	26
Western	2.000	39	19	6	14
Australia	5.993	47	23	7	17

Relationship between inoculum level and yield losses

Using data from multiple experiments conducted nationally, including those reported by Hollaway et al. (2013), yield losses in varieties with different levels of FCR resistance under varying FCR pre-plant risk categories were estimated for each zone within each of the three regions under three seasonal scenarios. Example yield loss estimates for zones within the Southern region are shown in Table 3. This highlights the important interplay between inoculum levels, seasonal conditions, and variety tolerance in determining losses due to FCR.

Table 3. Example estimated yield losses (%) for wheat cultivars with different reactions to Fusarium crown rot during different seasons in the presence of different pre-sowing inoculum levels based on risk categories in the Southern Region (zones 6 to 10)

Pre-sowing inoculum level	Variety reaction								
	Very susceptible			Susceptible			Moderately susceptible		
	Type of season[a]								
	Cond.	Int.	Non.	Cond.	Int.	Non.	Cond.	Int.	Non.
Not detected	0	0	0	0	0	0	0	0	0
Low	15	8	0	5	3	0	5	3	0
Medium	30	18	5	15	8	0	10	5	0
High	50	30	10	30	18	5	20	10	0

a: Cond. = Conducive season; Int. = Intermediate season; Non. = Non-conducive season.

Economic impacts of Fusarium crown rot

The average annual percentage yield loss and the economic value of such losses from FCR in each of the three regions and nationally were estimated (Table 4). The weighted average figures for loss (%) and total loss (A $ millions) takes into account actual levels of FCR inoculum measured in each region, the frequency of different seasons and the reaction of the cultivars grown to FCR.

Table 4. Estimated annual yield loss and value of loss due to Fusarium crown rot in three regions and nationally in Australia

Region	Loss (%)[a]				Total loss (A $millions)[a]			
	Cond.	Inter.	Non.	Weighted average[b]	Cond.	Inter.	Non.	Weighted average[b]
Northern	8.4	4.6	1.2	5.3	197	103	24	112
Southern	10.4	5.8	1.2	6.6	235	125	24	125
Western	10.4	5.8	1.2	6.6	264	141	43	167
Australia				6.2				404

[a] Cond. = Conducive season; Inter. = Intermediate season; Non. = Non-conducive seaso

[b] Seasonal loss weighted by frequency of the season types. For example, the frequency of occurrence of conducive, intermeidate and non-conducive varied from 0 to 70%, 10 to 30% and 10 to 90%, respectively in the zones that make up the southern region.

Discussion

The average annual loss from FCR was estiamted to be 6.2% worth $A404 million, which is considerably more than the 1.7% loss worth $A79 million estimated in 2009 (Murray and Brennan 2009). There has been an increase in cropping intensification and stubble retention practices across Australia since 2009 with the adoption of stubble retention practices being a major driver of increasing FCR levels (Simpfendorfer et al. 2019). Since the control of cereal cyst nematode in the Southern region (Vanstone et al. 2007), it has become possible to intensify cereal production. In the Western region, FCR has increased with tighter cereal rotations and stubble retention practices from an estimated $5 million in 2009. However, it is also possible that the methods used to assess yield losses in previous studies, which were based on opinions of a limited number of plant pathologists, potentially under estimated losses. The approach used in this study, based on unbiased measured levels of FCR inoculum in grower fields, provides a more robust assessment of disease losses.

Current recommendations in Australia for wheat growers are to test fields (e.g. PREDICTA®B testing) before sowing to identify those at risk of yield loss. Where yield limiting levels of FCR inoculum are present and it is economic to do so, rotate to non-cereal crops (Evans *et al.* 2003). However, where cereals are the only viable option, avoid varieties that are intolerant, plant early in the sowing window, avoid excessive application of nitrogen early in the season, use inter-row sowing to limit contact with previous standing cereal stubble and ensure adequate crop nutrition, especially zinc. Further research into the use of fungicide seed treatments within integrated management strategies and the breeding of wheat cultivars with improved tolerance is warranted.

Acknowledgements

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Wheat crown rot in Algeria: occurrence, distribution and pathogenicity of associated species and biological control option for disease management

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Summary

Crown rot (CR) is a worldwide wheat disease, affecting yield and quality. In Algeria CR was reported more than 20 years ago, however it was only recently that large surveys were carried out to investigate disease occurrence and distribution. Investigations carried out showed the presence of wheat crown rot in all the cereal-producing provinces surveyed in northern Algeria (humid-sub-humid and semi-arid bioclimatic stages). The analysis of symptomatic crowns showed that the main pathogen species associated with crown rot are *F. culmorum* (68%) *F. pseudograminearum* (10%) as well as *Microdochchium majus* (13%) and *M. nivale* (4%), where *F. culmorum* was the dominant species, and the presence of other species with less frequency. Pathogenicity of the main species associated with wheat crown rot was carried out using two inoculation methods, by seed inoculation where the pathogen effect was evaluated on the *in vitro* coleoptile growth rate of wheat seedling, and *in planta* test by soil inoculation to evaluate the pathogen crown rot indices. Results of the pathogenicity tests showed that *F. culmorum* and *F. pseudograminearum* isolates were both the most pathogenic by *in vitro* test inducing the highest percentage of coleoptile growth reduction whereas by *in planta* test, the *F. pseudograminearum* isolates induced the greatest crown rot disease indices. However, *Microdochium* isolates induced the lowest diseases indices by both inoculation methods. The biocontrol test carried out on wheat using the antagonist *Trichoderma* showed that the native isolates *T. atroviride* (Ta.09), *T. orientale* (To.15), *T. afroharzianum* (T af. 17 and T af. 37) and *T. gamsii* (T g. 39) significantly reduced disease severity compared to the uninoculated control (>82%). Similarly, it has been shown that *T. atroviride* induces metabolites associated with resistance to *F. culmorum* as total protein, polyphenols as well as antioxidant enzymes (peroxidases and catalase) in the three wheat varieties tested where the highest induction rate was recorded in wheat co-inoculated with *F. culmorum* and *T. atroviride* compared to the other treatment. Results obtained in the present study showed that native *Trichoderma* isolates are very promising for crown rot control and can be used as part of integrated disease management.

Introduction

Wheat (*Triticum* spp.) is the most cultivated cereal around the world under very diverse climatic conditions. In 2020, more than 760 million tons of this cereal were produced on 219 million hectares worldwide, for the same period, more than 3 millions tons of wheat were produced on 1.8 million hectares in Algeria (FAOSTAT 2022). In Algeria, wheat occupies a strategic place in the food system and in the national economy. Unfortunately the national production of wheat does not cover the needs of the population. Algeria can only meet 34 to 36% of those wheat needs. Wheat is subject to many abiotic (drought, salinity, unfavorable temperatures) and biotic stresses which limit production. Among the biotic stresses, crown rot (CR) is a major disease in most cereal growing regions worldwide (Liu and Ogbonnaya 2015). CR is a chronic problem where dry climatic conditions are present and when continuous wheat cultivation is adopted (Obanor and Chakraborty 2014). In Algeria, both conditions are found, wheat cultivation is concentrated mainly in semi-arid and arid zones (Boualal et al. 2007) and carried out continuously in most situations. Crown rot on wheat in Algeria was reported by Sayoud et al. (1999), however it was only recently that large surveys were carried out in the cereal provinces and reported diseases distribution and associated species (Moumen et al. 2013, Laraba et al. 2017a, Abdallah –Nekache et al. 2019). Many investigations worldwide reported important wheat losses in different area of wheat production (Smiley et al. 2005, Saremi et al. 2007, Hollaway et al. 2013, Chekali et al. 2013). So far, in Algeria, reliable data on yield losses due to crown rot are not available. Actually, the use of healthy seed coated with fungicide is an effective means of control but is generally limited to the early stages of the wheat cycle (Scherm et al. 2013). Many studies have also shown the benefits of non-host crop rotation and fallow in the crown rot management (Evan et al. 2010, Chekali et al. 2016). However, crop rotation can also have its limits economically (Kazal and Gardiner 2018). The use of resistant varieties is a key component in CR crown rot management. Although efforts and research are being undertaken to identify the genes involved in resistance to FCR, only a limited number of varieties with partial resistance have been identified, and there is no wheat cultivar that has been identified and released with resistance or full immunity (Wallwork et al. 2004, Kazal and Gardiner 2018, Al Ahmad et al. 2018). Otherwise, the application of natural antagonists by seed coating could reduce CR severity. Several works have been reported where *Trichoderma harzianum* reduced disease severity in the field (Roberti et al. 2000) and in the greenhouse (Dendouga et al. 2016). Recent studies have also shown that wheat co-inoculation with *T. gamsii* A5MH and *T. harzainum* Tr906 suppresses the *in-planta* abundance of the crown rot pathogen *F. pseudograminearum* and impacts the rhizosphere soil fungal microbiome (Stummer et al. 2022). In this conference paper, we present the current status of the occurrence and the distribution of wheat CR in the cereal provinces of Northern Algeria as well as the identity of the associated species which is a key step in the disease management. We present also data related to the antagonistic activity of some native *Trichoderma* species against *F. culmorum*, the main species associated with CR in Algeria, as well as the *Trichoderma* effect on biochemical parameters associated with resistance in wheat to *F. culmorum*. These investigations aimed to use these antagonistic agents within a disease management framework. Data presented in this conference paper are based mainly on previous published data.

Methods

Crown rot occurrence, distribution, associated species and pathogenicity

Surveys were carried out during April and May over two years 2014 and 2015. Nineteen (19) provinces in northern Algeria were surveyed for wheat sample collection with typical symptoms of crown rot (Figure 1). After isolation, identification of the pathogenic species was based first on morphological characters as described by Burgess et al. (1994) and Leslie and Summerell (2006). In order to confirm the morphological identification, a PCR (Polymerase Chain Reaction) was carried out by testing the isolates with species-specific primers for more details see Abdallah-Nekache et al. (2019). The pathogenicity tests of the main isolated pathogens were carried out by two inoculation methods by *in vitro* seed inoculation according to a modified method of Mesterhazy (1983), where aggressivity was expressed as percentage of coleoptile growth retardation compared to the uninoculated control seedling. Pathogenicity on the crown was assessed by soil inoculation carried out according to the protocol described by Demirci and Dane (2003).

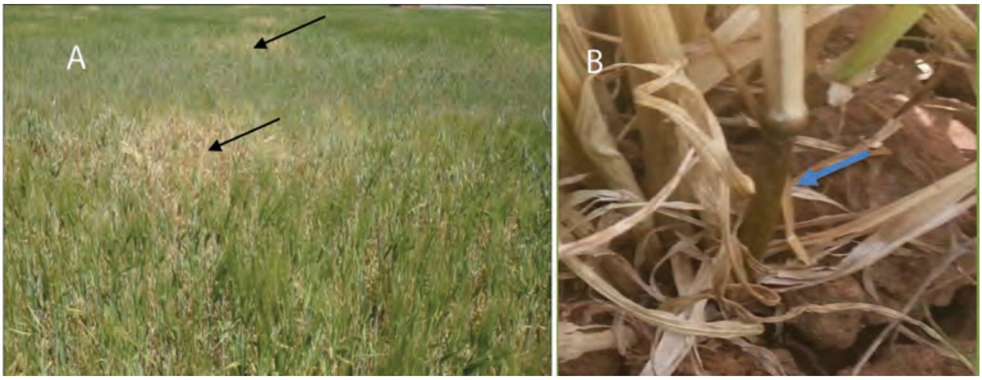

Figure 1. Wheat crown rot symptom as white head patches in the field (A), browning of the basal stem (B).

Biocontrol assay of *Trichoderma species* on wheat crown rot by seed treatment

Five native *Trichoderma* isolates, T a. 09 (*T. atroviride*), T o. 15 (*T. orientale*), T af. 17; T af. 37 (*T. afroharzianum)* and T g. 39 (*T. gamsii*) were selected based on strong inhibition of *F. culmorum* by *in vitro* test. Seed treatment by *Trichoderma* spp. isolates was performed according to the protocol described by Harman et al. (1989). The pathogen inoculation was done following the protocol described by Dermici and Dane 2003 as reported above. For more details see Chihat et al. (2021).

Investigation of the potential of *Trichoderma* to induce metabolites and antioxidative enzymes related to defenses mechanism on wheat against *F. culmorum*

The ability of *T. atroviride* (Ta.13) on the induction of total protein, and polyphenol as well as the antioxidative enzymes (peroxidase and catalase) on three wheat varieties durum wheat (Waha and Vitron) and bread wheat (Ain Abid) commonly cultivated in Algeria were evaluated. These varieties were chosen based on the fact that Waha and Ain Abid varieties showed the largest disease index reductions when wheat plants were co-inoculated by both *T. atroviride* and *F. culmorum* (unpublished data), and also on that cv. Vitron is a sensitive variety to crown rot under Algerian field condition. Chemical changes were evaluated in both the infection zone (basal part) and in leaves after 15 days post inoculation. Wheat seed treatment by *Trichoderma* was performed according to Harman *et al.* (1989) method, and wheat inoculation by *F. culmorum* was done following the protocol described by Dermici and Dane (2003). The experimental trial consisted of (1) the uninoculated control (2) wheat inoculated with *F. culmorum* alone (3) wheat inoculated with *Trichoderma* (Ta.13) alone and (4) the co-inoculated wheat with both Ta.13 and *F. culmorum*. For each treatment, there were (3) replicates performed, a replicate being a single pot (1-liter) in which 16 seeds were sown. Fifteen days after sowing, the plants (three-leaf stage) were pulled up and transferred into liquid nitrogen and were stored at −80°C until analysis. The analysis of the biochemical parameters mentioned above were done on two parts of each of the three sample plants: the basal part (collar and part of the root) and the apical part of the leaf of one plant randomly selected from each pot; for more details see Belhadj Benyahia et al. (2020).

Results

Crown rot occurrence, distribution, associated species and pathogenicity

Surveys carried out in northern Algeria have shown that crown rot was present in the 19 surveyed provinces of the humid, sub-humid and semi-arid bioclimatic stages of the central, eastern and western regions (Figure 2). Based on morphological criteria pathogen isolates were assigned to two fungal genera *Fusarium* and *Microdochium* with a preliminary identification of *Fusarium* species. *Microdochium* isolates were initially distinguished from *Fusarium* isolates mainly by the presence of annelledic conidiogenous cells instead of phialide and the absence of conidial foot cells, as reported by Samuels and Hallett (1983) and Hoshino *et al.* (2009).The use of species-specific PCR showed that *F. culmorum* (68%) is the dominant species, with the presence of *F. pseudograminearum* (10%) as an important species as well as *Microdochium majus* (13%) and *M. nivale* (3%), and other species with less importance. *F. culmorum* was the dominant species isolated from diseased crowns in most surveyed area in the different bioclimatic stages of the central, eastern and western area, while *F. pseudograminearum* was reported only in the semi-arid area. *M. majus* occurred in the semiarid bioclimatic locations of the east and west region and also found in the sub-humid central region. At the same time, *M. nivale* was present in the semi-arid eastern region and in the central sub-humid stage.

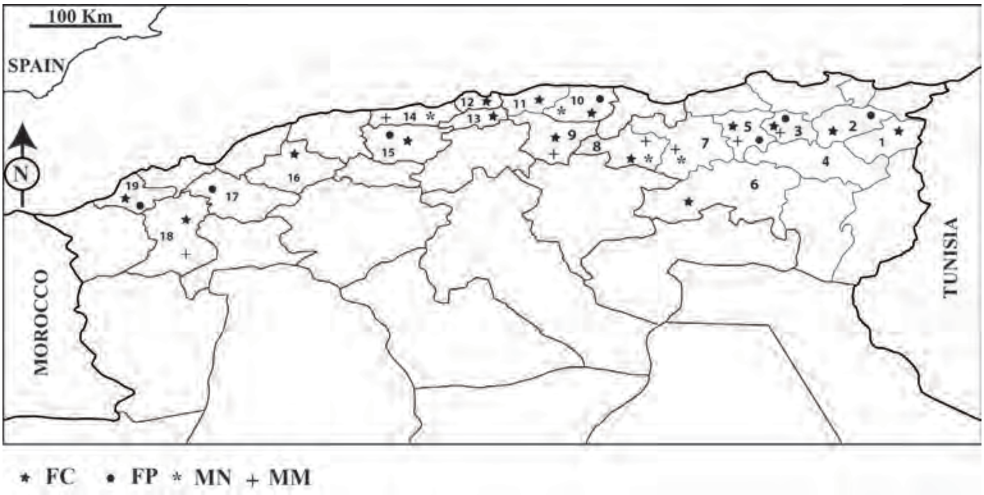

Figure 2. Map of Northern Algeria showing FCR distribution and different species (FC: *F. culmorum*, FP: *F. pseudograminearum*, MN: *M. nivale* and MM: *M. majus*) in the surveyed provinces during 2014 and 2015. 1 Souk Ahres, 2 Guelma, 3 Constantine, 4 Oum El Bouaghi, 5 Mila, 6 Batna, 7 Sétif, 8 Bordj BouArreredj, 9 Bouira, 10 Tiziouzou, 11 Boumerdes, 12 Alger, 13 Blida, 14 Tipaza, 15 Aïn El Defla, 16 Relizane, 17 Mascara, 18 Sidi Bel Abbes, 19 AïnTémouchent.

Among the pathogens isolates recovered during the survey, 10 isolates of *F. culmorum* collected from wheat ears (FCE), 10 isolates obtained from the crown (FCC) together with 10 isolates of *F. pseudograminearum* (FP) and 10 isolates of *Microdochium* spp. (M) were tested. Based on both inoculation methods by *in vitro* seed inoculation, and in *planta* test by soil inoculation *F. culmorum* and *F. pseudograminearum* were the most pathogenic while *Microdochium* isolates were the less aggressive. Data analysis of the disease indices average of the 4 groups of isolates (FCE, FCC, FP and M) classified them into two categories, the first containing three groups FCC, FCE and FP respectively which are considered to be the most pathogenic, and the second one includes isolates of *Microdochium* spp. It was also noted that there is no significant difference between the aggressiveness of the three groups FCC, FCE and FP ($p = 0.21$). However, on the crown interspecific variability of crown rot indices and significant difference ($p < 0.001$) between the four groups studied were detected. The most aggressive group was FP followed by FCC, then by FCE and the least aggressive group M. A significant difference was also noted between the two species *F. pseudograminearum* and *F. culmorum* ($p < 0.05$). A positive and significant correlation $r=0.70$ ($p<0.05$) between the disease indices assessed by the *in vitro* seed inoculation and the soil inoculation method was noted for the FCC isolates group of *F. culmorum*.

Wheat protection by *Trichoderma* seed treatment

The biocontrol assay carried out on wheat plant showed that *T. atroviride* (Ta.09), *T. orientale* (To.15), *T. afroharzianum* (T af. 17 and T af. 37) and *T. gamsii* (T g. 39) performed well against *F. culmorum* the main crown rot pathogen of wheat in Algeria. This finding is based on the significant decrease in disease severity compared to the control (>82%). Data have also shown that *T. atroviride* Ta.09 recorded the highest percentage of disease reduction (97.28%)

Biochemical parameters associated with defense mechanism on wheat against *F. culmorum*

Data has shown that *T. atroviride* induced important biochemical parameters related to disease resistance, such as total phenol accumulation, catalase, and peroxidase activities. This induction was systemic and depended on the wheat variety. A significant difference for each studied parameter between varieties for each treatment was recorded, with levels being lowest in uninoculated control plants, followed by plants inoculated with *F. culmorum* alone, plants inoculated with *T. atroviride* (Ta.13) alone, then plants inoculated with both *T. atroviride* and *F. culmorum*.

Discussion

Wheat crown rot occurs in all cereal provinces surveyed, in the different bioclimatic locations *F. culmorum* was the dominant species isolated from wheat crowns in most surveyed regions. This result matches those obtained in previous studies carried out in other Mediterranean countries such as Tunisia (Rebib et al. 2014, Oufensou et al. 2019), Morocco (El Yacoubi et al. 2012), Türkiye (Tunali et al. 2008, Shikur-Gebremariam et al. 2018), Italy (Scherm et al. 2013), and other countries such as Iraq (Matny et al. 2012, Iran (Eslahi 2012) and New Zealand (Bentley et al. 2006). *F. pseudograminearum* was recorded in semi-arid climate in the east, center, and west of Algeria. This species was reported mainly in semi arid area of Tunisia (Kammoun et al. 2009). *F. pseuodgraminearum* is the main causative agent of crown rot in Australia where wheat is cultivated mainly under relatively warm and dry conditions (Chakraborty et al. 2006, Obanor and Chakraborty 2014), in New Zealand (Bently et al. 2006), and in Iran (Saremi et al. 2007). Recently, it has been reported as the main agent of wheat crown rot in China (Zhou et al., 2019). Data recorded showed that *M. majus* was more frequent on wheat crowns than *M. nivale*, a similar situation had been reported in previous studies where *M. majus* was more predominant on wheat stem bases and heads than *M. nivale* (Parry et al. 1995), while *M. nivale* was predominant on rye (Miedaner et al. 1993). It is worthy to note that *M. majus* and *M. nivale* were isolated exclusively from the wheat crown in Algeria, however *M. nivale* was reported as the main species associated with FHB in Tunisia by Kammoun et al. (2009), and *M. majus* was isolated from wheat seeds in Morocco (Saoudi et al. 2018). Recently *F. algeriense* a new species has been reported and described on durum wheat in Algeria, this species caused moderate crown rot symptom (Laraba et al. 2017b). *F. algeriense* was later reported in other cereal growing area outside of Algeria on bread wheat in Kazakhestan (Özer et al. 2019) and Krygstan (Özer et al. 2022).

Similar results have been reported in previous study about the high pathogenicity of *F. culmorum* and *F. pseudograminearum* on the wheat crown compared to other species with less aggressivity (Shikur-Gebremariam et al. 2018; Dehghanpour-Farashah et al. 2020). *M. majus* and *M. nivale* were less aggressive on wheat crowns. Contrary to the results obtained in the present study, Dermici and Dane (2003) found that *M. nivale* isolates were very aggressive on the wheat crown. In cold to temperate areas, both species *M. nivale* and *M. majus* cause the pink snow mold, also known as Microdochium patches on turf, forages grasses, and cereals (Tronsmo *et al.* 2001, Abdelhalim *et al.* 2020). At warmer temperatures, they also induce crown rot, leaf blotch, and FHB (Pancaldi et al. 2010, Nielson et al. 2010, Abdallah-Nekache et al. 2019).

In wheat crown rot biological control assay, the highest percentage of disease reduction was recorded by *T. atroviride* (T a. 09) and *T. afroharzianum* (T af. 17). Based on these results, Ta. 09 and T af. 17 appear to be the best isolates to control the wheat crown rot caused by *F. culmorum*. Comparable results on wheat crown protection against *F. culmorum* have been reported in previous studies. Dendouga et al. (2016) reported a significant reduction in disease index (>70%) induced by two isolates of *T. harzianum* and one isolate of *T. viride* against *F. culmorum*. In addition, Roberti et al. (2000) showed that *T. harzianum*, *T. atroviride* and *T. longibrachiatum* were effectives in reducing the severity of wheat crown rot caused by *F. culmorum*. Wheat seed treatment with *T. atroviride* (Ta.13) induced changes in protein and phenolic contents as well as on the catalase and peroxidase activities of the resultant wheat plant. This induction varied more or less considerably from one variety to another and from one part of the plant to another (basal part and leaves). In general, maximum induction was recorded in the Waha variety, followed by Ain Abid, when plants were co-inoculated by both Ta.13 and *F. culmorum* versus *F. culmorum* only. At the same time, lowest levels of these factors occurred in the Vitron variety. Actually Waha and Ain Abid varieties showed the largest disease index reductions when wheat plants were co-inoculated by both *T. atroviride* and *F. culmorum* (unpublished data), while cv.Vitron is a sensitive variety to crown rot. Recent investigation reported that *Trichoderma* spp. have the ability to establish an avirulent relationship with the plant and to elicit a durable defense (priming) which strengthens and accelerates the plant defense response (Monte and Hermosa 2022). Also, it has been shown that in addition to a direct biocontrol, *T. atroviride* was able to induce systemic resistance without establishing any contact with the pathogen, and that tomato progeny of the priming plant inherited resistance (Medeiros et al. 2017). Using antagonistic microorganisms as seed treatment can allow a long protection compared to chemicals which the effectiveness is very limited to early cycle of the plant, and would be a promising way to control soil borne pathogens which are difficult to manage.

Conclusion and perspectives

A global knowledge of wheat crown rot in Algeria still requires work by prospecting the regions which have not yet been surveyed in Northern Algeria and also the southern regions where the cultivation of wheat is practiced under pivot and at the same time in rotation with maize. In this area the climatic conditions are different from those in the north. The occurrence of the disease as well as the associated species deserves to be investigated. Crown rot associated species monitoring is important, especially with the impact of climate changes in recent years. Data obtained in the present study have also shown that native species of *Trichoderma* isolated from different ecosystems and regions were effective in reducing crown rot disease indices, hence the possible application in different bioclimatic stages of the country where wheat is grown. Host resistance is a key factor on disease, and currently we are collaborating with the Soil Borne Pathogen Program of CIMMYT- Türkiye by testing bread wheat lines developed by the program to crown rot under our local conditions. It is worthy to note that the presence of FHB in humid sub-humid area of Algeria where crown rot is also present can be a source of additive inoculum in the soil. Consequently, in crown rot management program we should take into consideration the presence of FHB while screening for resistance, chemical or other optional controls.

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An overview of soil-borne pathogens of cereals in Tunisia

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Summary

This paper provides an overview of the research activities carried out from 2010 at the Plant Protection Laboratory of the National Institute of Agronomic Research of Tunisia on soil and residue-borne diseases of cereals: monitoring and management under the Tunisian farming systems. Soil and residue- borne diseases including Fusarium foot rot (caused by *Fusarium* spp.), eyespot (caused by *Oculimacula* spp.) and take-all (caused by *Gaeumannomyces* spp.) are a major constraint to cereal productivity and quality. These pathogens can be challenging to identify and their importance is likely to be underestimated in many farming systems because of their non-specific symptoms of infection. A lack of genetic and/or chemical control options make their management much more challenging compared with foliar diseases. To investigate their incidence and distribution, surveys were conducted at permanent sites and farmers' fields. Management trials were set up in experimental stations and farmers' fields under natural infestation or inoculation. The findings highlight the wide distribution of soil-borne diseases across the cereal growing area of Tunisia and emphasis the need for efficient pre-sowing diagnostic tools for risk estimation and for establishing appropriate disease management strategies. The impact of different management strategies including seed treatment, rotations, genetic resistance, and biological control will be discussed. More effort should be dedicated to raising awareness of the impact of soil-borne pathogens among different stakeholders for monitoring these diseases and preventing yield and quality losses of cereal production, especially in the context of climate change, and the increasing pressure on demand for cereals.

Introduction

Cereal production has always been an important component of Tunisian agriculture. The average per capita consumption of cereals in Tunisia is 184 kg per year (ONAGRI 2016). They are grown on approx. 1 M ha of the agricultural land, about 63% of farmers are smallholders, each with land areas of less than 10 ha (Bachta 2011). Cereals are sown between October and December in most of the rain-fed farms. The rainfall in the grain belt varies from more than 600 mm in the northern region to less than 300 mm in the southern region. Cereal production may be affected severely by plant pathogens especially in short rotations and monoculture, which are common practices in Tunisia. Soilborne pathogens are among the most underestimated of the factors which affect crop production. They cause important diseases, resulting in stand reductions, rotting of root, crown, sub crown, and lower stem tissues of seedling and mature plants, white-heads and grain yield and quality losses. Some pathogenic species are also responsible for mycotoxin accumulation in cereal tissues that are harmful for food and feed. Soil-borne diseases are often referred to as intractable diseases, due to their broad host ranges, difficulties of targeting the pathogen population in the soil with chemistry and the slow progress when breeding for genetic resistance to infection. In the field they often occur as mixed populations and their combined effect on crop production is likely variable across the diverse cropping environments. In Tunisia, continuous efforts are dedicated to foliar diseases especially in the national breeding program, whereas very little work has been focused on soil-borne diseases in the past. This work focuses on the dominant soil and residue-borne diseases: Fusarium foot and root rot (FFRR), take-all (TA) and eyespot (ES).

FFRR has been reported since the 1970's in Tunisia (Gargouri et al. 2001). The different cereals, including wheat, barley and oats, can be infected with durum wheat being the most susceptible (Wallwork et al. 2004). The disease is caused by a complex of *Fusarium* species. The most important are *F. culmorum* and *F. pseudograminearum* (Smiley and Patterson 1996; Paulitz 2006) that may vary in importance according to the geographical areas and the agronomic factors.

These two pathogens produce lesions on the coleoptiles, roots, and sub crown internodes of host plants, and cause browning of the stem bases at or near the soil surface, from soil- or residue-borne inoculum. Infection is favoured by wet conditions shortly after seeding, and disease severity increases and yield reductions become significant when infected plants are subjected to water stress and/or high temperatures late in the growing season (Paulitz et al. 2002; Chekali el al. 2016). These species are able to biosynthesize mycotoxins in grains but also in stem tissue that have strong acute and chronic toxicity.

Take-all of cereals caused by the soil-borne fungal pathogens, *Gaeumannomyces tritici* and *Gaeumannomyces avenae,* is also a common disease worldwide. Gt causes take-all of wheat, barley, and rye and is the most economically important take-all pathogen, whereas Ga is less common and can also infect oats (Wallwork et al. 2004). Take-all is more common where cereals are grown under moist conditions, which enhances root infection during the winter and early spring. Symptoms are most obvious near heading and include poor tillering, blackened roots and crowns, premature ripening, and white heads with few kernels. Root systems of severely diseased plants may be sparse and brittle. Late-season drought stress causes the entire diseased plant to ripen prematurely further reducing yield (Wallwork et al. 2004).

Eyespot, caused by *Oculimacula yallundae* and *O. acuformis*, is an important fungal disease of the stem base of cereals grown in temperate regions (Lucas et al. 2000; Crous el al. 2003) Eyespot forms lesions on the leaf sheaths and culms near the soil level, and their elliptical shape gives rise to the name of the disease, which is also known as strawbreaker foot rot. Infection takes place at the lower stems of plants, resulting in stem breakage, lodging and yield reduction. These pathogens have a wide host range including cereals and grass species (Lucas et al. 2000). Wheat, barley, rye, oats, and other related grasses can be affected, with wheat being the most susceptible (Chapman et al. 2008).

Aerial symptoms caused by these diseases are non-specific and may be confused with other stressors including abiotic stressors such as drought. Their effect often goes unnoticed until the end of the season and there are few in-season management options. In this work we report on the occurrence of the soil-borne diseases of cereals in the different climatic regions of Tunisia, and the impact of management strategies to control these diseases.

Methods

Surveys

Wheat, barley and oat fields were surveyed randomly from 2010 to 2022 across four different climatic zones of Tunisia: the humid, sub-humid, upper semi-arid and lower semi-arid. Permanent sites where the previous crops were recorded were surveyed during three cropping seasons. The incidences of TA and ES were evaluated as the percentage of plants with characteristic symptoms of each disease, whereas the incidence of FFRR was evaluated as the frequency of isolation of the dominant *Fusarium* species.

Pathogen identification and detection in the soil

The pathogens were isolated from diseased stems and roots on ¼ strength PDA. The identification was based on morphology and PCR amplifications using specific primers for each species (Nicholson et al. 1997; Rachdawong et al. 2002; Schilling et al. 1996). Sequencing of the internal transcribed (ITS) region was used to confirm the identification of *Gaeumannomyces* spp. and *Oculimacula* spp., whereas sequencing of the translation elongation factor (EF1-α) gene was used for *Fusarium* spp.

For pathogen detection in the soil, samples were collected to a depth of 100 mm prior to sowing following the SARDI protocol (Ophel-Keller et al. 2008). Sub-samples were sent to SARDI for qPCR as part of the PreDicta B soil testing in which levels of pathogenic fungi are related to disease risk. Quantitative polymerase chain reaction assays using rDNA (*Taq*Man) probe sequences specific to 14 pathogens were applied to the total DNA extracted from the soil (Ophel-Keller et al. 2008).

Potential yield and quality loss

Yield loss from foot and root rot and eyespot of wheat were estimated under natural infestation in farmer's fields following the individual tiller method of Dodman and Wildermuth (Dodman and Wildermuth 1987). Inoculated versus non inoculated trials were also set up to estimate the potential yield and quality loss caused by *F. culmorum* in wheat. Foliar fungicide application was used to estimate the potential yield loss caused by eyespot in a highly infested farmer's field.

To evaluate the impact of soil-borne diseases on grain quality, test weight (TW), percentage of yellow-berry, SDS test and gluten index were measured for grains collected from *F. culmorum* inoculated and non-inoculated trails as well as from fields with different levels of infestation by the soil-borne pathogens. In addition, potential mycotoxin accumulation from *Fusarium* species was estimated in wheat straws and grains in inoculated field trials.

Management

Seed treatment: the efficiency of seed treatments was evaluated in-vitro, in the greenhouse and in the field under inoculated or non-inoculated conditions and under two rotation systems (wheat/wheat, wheat/faba bean) using different fungicides including: fludioxonil+difenoconazole; prochloraz+triticonazole;

difenoconazole and sedaxane+fluodoxinil. Non treated seeds were used as control. *In-vitro* and in the greenhouse, the emergence, plant and root length, the incidence and the severity of the disease were evaluated. In the field, the agronomic performance including emergence, tillering, number of ears/m^2, thousand kernel weight (TKW) and grain yield were measured. The incidence and the severity (stem discoloration and white heads) of the disease were evaluated at seedling-early tillering stage then at dough stage. Mycotoxin accumulation in the stem tissue was also measured.

Host range: grass weeds were collected from cereal and non-cereal fields. Visual assessment and isolation of pathogenic fungi were carried out on roots and stems.

Conservation on residue. The population *F. culmorum* was monitored in the residues of four cereal crops (oat, barley, durum wheat and bread wheat) stored at two depths of soils in two climatic areas (sub-humid and semi-arid). The survival of *F. culmorum* on the crop residues was estimated by classic isolating and quantitative real-time polymerase chain reaction (qPCR) at four dates over a 29-month period.

Rotation. Rotation trials were conducted in experimental stations and farmer's fields. Faba bean, fenugreek and oat were used as break crops following farmer's practices. Data on the incidence and severity of the diseases were collected from these trials as well from the surveys. Inoculum level in the soil was estimated in some of the trials.

Foliar fungicide application. Field experiment were set up to test the effect of fungicide treatments (epoxiconazole, epoxiconazole +metrafenone, metrafenone, prothioconazole+tebuconazole, tebuconazole +prochloraz , cyprodinil) on eyespot. Fungicides were applied at stem elongation. The incidence and severity of the disease and percentage of lodging were evaluated at grain filling. Grain yields was also assessed.

Genetic resistance. Several lines from a specific crown rot international CIMMYT nursery and from the national durum and bread wheat breeding program were screened using a modified CIMMYT protocol under greenhouse conditions, against *F. culmorum* and *F. pseudograminearum* isolates over 3 years.

Biological control. Assays of wheat seed coating with *Trichoderma harzianum* strains, rhizospheric and endophytic bacteria were conducted to evaluate the direct antagonist activity against *F. culmorum* and the indirect bio-promotion of plant growth *in-vitro* and in the greenhouse.

Results and discussion

Surveys conducted across years and in different climatic regions indicated that at early stages, *Fusarium* and *Pythium* species were the dominant pathogens, later *Fusarium*, *Gaeumannomyces* and *Oculimacula* species were the most frequent. In many fields more than one pathogen was recovered from cereal crop. The incidences of the diseases varied among years and climatic regions. Fusarium foot and root rot is by far the most widespread disease occurring in more than 70% of the fields each cropping season with incidences reaching 100% in many fields (Khemir et al. 2020, Guermech et al. 2022). The highest incidences were recorded in the semi-arid regions. However, recent surveys showed an increase in the incidence of FFRR in more humid regions. Eyespot and take-all are more restricted to wet areas where annual precipitation is higher than 500 mm (Gargouri et al. 2020, Gargouri et al. 2021) which in agreement with other studies (Kwak and Weller 2013). It should be noted that these surveys focused only on dryland crops. Take-all and eyespot diseases may be more severe and responsible for considerable yields loss in irrigated crops (Paulitz et al. 2010). The surveys also revealed that the number of fields affected with eyespot as well as the incidence and severity of the disease increased significantly over a 5-year period. In South Australia, eyespot has been causing increasing problems, the change being attributed mainly to changes in farming systems (Evans and Wallwork 2019) An increase in eyespot incidence has also been observed in central and northern Italy (Covarelli and Nicholson 2002, Innocenti et al. 2013).

Identification analysis demonstrated that among *Fusarium* pathogenic species, *F. culmorum* was the most important species associated with FFRR similarly as in Europe. Whereas, *F. pseudograminearun* the dominant species in the Pacific North West and Australia is rarely isolated being more restricted to arid regions (Gargouri et al. 2011). For eyespot, only *O. yallundae* was recovered from characteristic symptoms so far (Gargouri et al. 2021). In other regions such as Eastern Europe, both species *O. yallundae* and *O. acuformis* coexist. *G. tritici* was also the only species isolated from blackened root tissue. These results were further confirmed by quantitative PCR analysis of soil samples. Among the 7 wheat fungal pathogens analysed, two were not detected in the soil samples (*G. avenae* and *Rhizoctonia solani* AG8), two were infrequent (*F. pseudograminearum, Bipolaris sorokiniana*) and the most common were *F. culmorum, G. tritici*, and *Pythim* spp. clade F**.** Globally, all plots surveyed were infested by at least three fungal pathogens and/or nematodes and up to eight were detected in the same sample. More than 50% of soil samples analyzed indicated a level of medium to high risk for at least one disease. There was also a positive correlation between the level of inoculum before sowing and the incidence of the disease the year after (Khemir et al. 2017).

Globally these pathogens induced their respective disease on all cereal crops including durum wheat, bread wheat, barley and oat, with durum being the more susceptible. All commercial varieties were susceptible compared to resistant lines under inoculated conditions. Assays of screening for resistance suffered from the lack of correlation between greenhouse and field conditions and reproducibility among years (Gargouri et al. 2009).

These pathogens were also isolated from grassy weeds such as *Lolium* sp., *Phalaris* sp., *Hordeum* spp. and *Avena* sp. collected from legume crops which negates their value as break crops (Gargouri et al. 2013).

The damage caused by these diseases has not been extensively studied previously in Tunisia. Assays conducted in farmers' fields and under inoculated conditions showed that FFRR reduced wheat yield as much as 30% with a mean of 10% (Gargouri et al. 2007), and inoculation induced yield losses up to 40% over that caused by the native pathogen flora (Chekali et al. 2013, Guermech et al. 2022). Eyespot fungicide application in naturally infested wheat fields increased the grain yield by up to 25% (personal data). Yield losses however depend on seasonal conditions, especially during grain filling. The damage caused by these diseases may be further accelerated in areas where water stress and monoculture practices dominate. Global warming is expected to be particularly intense in the Mediterranean region (Varotsos et al. 2021), thereby suggesting that drought will have a growing impact on wheat production and quality in MENA region during the years ahead.

Soil-borne pathogens may affect also the quality parameters such as a decrease in starch content and an increase in protein content, yellow berry percent and pasta cooking time (Sfayhi et al. 2022). In addition, some *Fusarium* species may be responsible for mycotoxin accumulation in wheat stem tissue. High concentration levels of deoxynivalenol were detected in wheat stems in inoculated fields (Guermech et al. 2022). One of the features of the Tunisian farming system is that sheep owners have the right to graze on cereal residue. In the dry areas the baled straw may be even more valuable than the grains. Whether mycotoxin accumulation in residue may affect animals under Tunisian conditions needs further investigation. Translocation of DON to the grain as reported in our studies may further decrease grain quality (Guermech et al. 2022).

In terms of disease management, introducing a one-year break crop such as faba bean or fenugreek resulted in reduction of take-all and FFRR incidence of up to 70% and severity up to 80% (Gargouri et al. 2020, Sfayhi et al. 2022). This was confirmed by the significant decrease of inoculum level of *F. culmorum* and *G. tritici* in the soil. The effect of the previous crops was also demonstrated for FFRR from the survey conducted in permanent sites over three years. The benefit of the break crop to control eyespot was controversial. The survival of *F. culmorum* was studied under different climatic conditions, on different cereal residues and at two depths. Isolation and qPCR revealed that there was a significant decrease in inoculum levels after 3 months. Neither isolation nor qPCR could detect the pathogen after 22 months (Khemir et al. 2020). Thus, rotation with a grass-free break crop is a key strategy to control these soil-borne diseases.

The benefit of seed treatment to reduce fungal growth and disease incidence and severity and to improve plant growth was clearly demonstrated *in-vitro* and in the greenhouse. In the field, these findings were confirmed especially under high infestation conditions (Zarra et al. 2017). As global awareness about environmental pollution increases, bio-management strategies are becoming popular methods. Seed coating with strains of *T. harzianum* (Kthiri et al. 2020) and *Bacillus* spp. and *Pseudomonas* spp. (Mnasri et al. 2016) showed promising results for improved wheat production and protection under field conditions due to both direct antagonist activity and the indirect growth promotion.

Soil-borne pathogens are still not widely recognized as a limiting factor for cereal production in Tunisia. Raising awareness among different users and more effective training and extension are crucial for monitoring those diseases and preventing yield and quality losses in cereal production. More effort should be dedicated to implement a multidisciplinary network at the national and regional level for a more holistic approach to the management of diseases caused by soil-borne pathogens taking into account soil health globally, especially in the context of climate change, and the increasing pressure on demand for cereals.

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Research on fungal soil-borne diseases on wheat in Iran

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Summary

Wheat is the most important crop in Iran and is attacked by a number of fungal soil-borne pathogens (FSBP) that cause wheat yield losses every year. In Iran, research on the diseases including identification of causal agents, as well as evaluation of cultivars and genotypes to find resistant ones and the effect of antagonistic agents for biological control of FSBP, began in the 1990s. The results of various research have shown that some different fungi and fungi-like organisms are associated with root and crown rot of wheat. Among them, the most important genera causing major losses are *Gaeumannomyces*, *Fusarium*, *Bipolaris* and *Rhizoctonia*. It was revealed that some cultivars and lines have partial resistance against some FSBP. Also, the use of microbial antagonists against FSBP has had varying results. Although in Iran, some useful research has been done, there is a wide gap between what has been done and what should be done to achieve suitable management of soil-borne fungal diseases of wheat. This article reviews research conducted on FSBP in Iran and the research perspectives to fill the existing gaps.

Introduction

Wheat is the most important crop in Iran and is attacked by a number of fungal soil-borne pathogens (FSBP) that cause wheat yield losses every year. In Iran, research on the diseases including identification of causal agents, as well as evaluation of cultivars and genotypes to find resistant ones and the effect of antagonistic agents for biological control of FSBP, began in the 1990s. The results of various research have shown that some different fungi and fungi-like organisms are associated with root and crown rot of wheat. Among them, the most important genera causing major losses are *Gaeumannomyces*, *Fusarium*, *Bipolaris* and *Rhizoctonia*. It was revealed that some cultivars and lines have partial resistance against some FSBP. Also, the use of microbial antagonists against FSBP has had varying results. Although in Iran, some useful research has been done, there is a wide gap between what has been done and what should be done to achieve suitable management of soil-borne fungal diseases of wheat. This review was prepared with the aim of informing about research that have been done on FSBP and the research needed in the future to fill the gaps.

History

Incidence of fungal soil-borne diseases in wheat farms in extensive regions, and on the other hand increase of plant pathologists in agricultural research centers and universities have resulted in conducting more investigations on these diseases in Iran since 1990s (Foroutan et al. 1989, Foroutan et al. 1995). At first, research was concentrated on the identification of the causal agents. Then the management of the diseases was pursued by evaluating the resistance of wheat cultivars and genotypes (Foroutan et al. 2002, Mohammadi kohnehshahri et al. 2019, Razavi et al. 2017) , as well as testing the effectiveness of antagonistic agents for biological control (Khezri 2017, Zafari et al. 2008).

To estimate percentage of FSBP-infected wheat plants, wheat fields were sampled randomly, and non-random sampling was used for monitoring and identifying possible species (Safaee et al. 2008). General and semi-selective culture media (Singleton 1992) were used to isolate FSBP. Recognition of the fungi was completely based on morphological criteria of anamorph and/or teleomorph in primary research and then molecular methods gradually combined with them to achieve more accurate diagnosis. It has been demonstrated that a lot of species of fungi and fungi-like organisms cause root and crown rot in wheat-growing regions of Iran. Also on the basis of pathogenicity tests and frequency of isolation, it was revealed that the genera inducing major losses to wheat production are *Gaeumannomyces*, *Fusarium*, *Bipolaris* and *Rhizoctonia* (Amini et al. 1998, Amini 2006, Arjmandian and Darvishnia et al. 1998, Babadost 1995, Darvishnia et al. 2006, Eskandary et al. 1998, Foroutan et al. 1998, Golzar and Ershad 1994, Kazemi et al. 2008, Mansoori 1995, Ravanlou and Banihashemi 1999, Safaee et al. 2000, Safaee *et al.* 2000a, Safaee 2004, Safaee et al. 2012, Saremi 2004, Zare and Ershad 1997, Zamanizadeh and Foroutan 1993).

Testing wheat cultivars and genotypes to appraise their resistance to FSBP has been conducted in greenhouse and/or field conditions (Mahdavi et al. 2022, Razavi et al. 2017) by adding the pathogens to pot or field soil then cultivation of wheat seeds into infested soil. Effectiveness of microbial antagonists was evaluated in laboratory and/or greenhouse conditions (Hashemi et al. 2013, Khezri 2017, Zafari et al. 2008).

The major research that have been done on each of the above genera related to wheat plants in Iran are mentioned as follows:

***Gaeumannomyces graminis* var. *tritici*:** *Gaeemannomyces graminis* var. *tritici* (*Ggt*) was reported from Mazandaran province (Foroutan et al. 1989) then it was isolated from root, crown and foot of wheat in the other parts of the country (Arjmandian and Rohani 1998, Fassihiani 1999, Ghalandar et al. 2000, Kazemi et al. 2008, Safaee et al. 2010). Crop rotation, fertilizer-balanced nutrition, using ammoniacal and slow-release forms of nitrogen in soil, biological control and resistant cultivars have been suggested to manage take-all disease (Paulitz 2010). Foroutan et al. (2002) evaluated some gramineous plants against Ggt and indicated that *Secale cereale* and *Lolium rigidum* were immune. *Alopecurus myoduroides* and *Avena fatua* were resistant and, *Horeum marinum* and *Phalaris minor* showed moderate resistance. *Triticum aestivum* and *H. vulgaris* were susceptible. Mohammadi Kohnehshahri et al. (2019) tested some wheat cultivars against *Ggt* and found that Marvdasht, Parsi, and Aflak were to some extent tolerant and Sardari, Sivand, Shiraz, and Alvand were identified as susceptible cultivars.

Some antagonists have been evaluated to biocontrol of *Ggt* in laboratory and/or greenhouse conditions. Zafari et al. (2008) evaluated biocontrol effects of nine *Trichoderma* isolates including *Trichoderma virens* T65, *T. virens* T90, *T. virens* T96, *T. virens* T122, *T. koningii* T77, *T. koningiopsis* T149, *T. brevicompactum* T146, *T. viridecens* T150 and two commercial bioproducts Trichodermin B and Subtilin against take-all pathogen in the greenhouse condition. Their results showed that isolates T96, T65, mixture of eight isolates and Trichodermin B were more effective than other treatments to control the disease and reduced 25 to 55% disease severity and increased 27 to 59% dry weight of shoots and 23 to 58% dry weight of roots compared with control (plants inoculated by *Ggt* and treated by *Trichoderma*). Disease reduction due to seed coating method was more than soil treatment applications. It is mentionable that shoot and root dry weight of all treatments was significantly lesser than healthy plants (non-inoculated and non-treated plants). In another research, potential of 27 bacterial antagonistic strains of *Bacillus subtilis* was evaluated for biocontrol of *Ggt* in the laboratory and greenhouse conditions. In dual culture experiment, all strains had inhibition zones against growth of the pathogenic fungus and they prevented fungal growth between 39.48-96.29%. Volatile metabolites of some strains reduced fungal growth up to 87.62% while for some others was less than 20%. Eleven strains with different potential in antagonistic effects and biofilm formation were selected for the possibility of disease biocontrol in the greenhouse conditions. All 11 strains decreased the disease between 20-100% (Khezri 2017).

***Fusarium* spp.:** Many *Fusarium* species have been isolated from all wheat growing areas of Iran (Amini et al. 1998, Amini 2006, Arjmandian and Darvishnia et al. 1998, Babadost 1995, Darvishnia et al. 2006, Eskandary et al. 1998, Foroutan et al. 1998, Golzar and Ershad 1994, Mansoori 1995, Ravanlou and Banihashemi 1999, Safaee et al. 2000, Safaee et al. 2000a, Safaee 2004, Safaee et al. 2012, Saremi 2004, Zare and Ershad 1997, Zamanizadeh and Foroutan 1993). Safaee et al. (2012) by means of pathogenicity tests in greenhouse condition revealed that among *Fusarium* species isolated in western Iran, *Fusarium culmorum* and *F. pseudograminearum* had the highest disease severity (DS), *F. acuminatum, F. avenaceum*, *F. crookwellense*, *F. proliferatum* and *F. udum* had medium DS and that of *F. nygamai, F. reticulatum and, F. merismoides* was the least amount. The species that could not cause disease were *F. equiseti, F. lateritium, F. moniliforme*, *F oxysporum, F. sambucinun, F. semitectum, F. solani* and *F. tricinctum.*

A research was conducted to evaluate resistance level of 70 cultivars/advanced lines of bread wheat and durum wheat obtained from CIMMYT, SPII and DARI, to a mixture of five *F. culmorum* isolates in Karaj and Kermanshah locations under field conditions. In addition, the reactions of these genotypes were evaluated against *F. pseudograminearum* and *F. culmorum* at the seedling stage in greenhouse conditions. The results showed that the cultivar Burbot-6 from CIMMYT and advanced line C-87-18 from SPII had a very good level of resistance to both fungal species under field and greenhouse conditions in both locations. These genotypes have the potential to be used directly by farmers in the infected areas of Iran or incorporated into the breeding programs at the national or international level (Razavi et al. 2017).

***Bipolaris* spp.**: The genus *Bipolaris* has several species among which *B. sorokiniana* is distributed worldwide and is an important pathogen of wheat in many regions, causing root rot, leaf spots, seedling blight, head blight and kernel blight (Stein 2010).

Bipolaris spp. on wheat crop mainly cause common root rot in Iran conditions (Ershad et al. 1996, Darvishnia 1998, Safaee et al. 2000b, Safaee 2004). The results of a research in Kermanshah province demonstrated that the percentage of diseased plants was 0-24% whose average was 3.2%. Forty three isolates belonging to the form genus *Bipolaris* were isolated including form species *B. sorokinina*, *B. cynodontis* and *B. spicifera*. All three form species were pathogenic under greenhouse conditions. Disease severity and frequency of *B. sorokinina* were more than the others. Also disease severity of *B. cynodontis* and *B. spicifera* were the same and their differences compared with the control were statistically significant (Safaee et al. 2008).

Samiei et al. (2008) tested the resistance reaction of 15 wheat cultivars and lines against *B. sorokinina* in greenhouse condition. They found that cultivars Sabalan, Zarrin and Niknejad were the most resistant in comparison to the other cultivars. Cultivars Maroon, Bakkras-roshan and Kavir were more susceptible. Pishtaz, Alvand, Gaspard, Alamoot, Darab2, Marvdasht cultivars, C-78-14 and M-79-6 lines were graded between these two groups.

The resistance levels of 66 bread and durum wheat genotypes against *B. sorokiniana, F. pseudograminearum* and *F. culmorum* were evaluated in greenhouse condition. The results showed that 11 genotypes were moderately resistant to diseases caused by *B. sorokiniana* isolates and its combination with *F. pseudograminearum* and *F. culmorum* Among these genotypes, three genotypes C-87-11, GA951079-3-5 / Neuse and 05899G01-2 were resistant to the combination of all diseases caused by these three fungal species (Mahdavi et al. 2022).

Hashemi et al. (2013) appraised the biocontrol potential of *Glomus fasciculatum*, *Pseudomonas fluorescens* Sh4 and a mixture of them for controlling *B. sorokiniana* in greenhouse condition. Their results revealed that the presence of *G. fasciculatum*, *Pseudomonas* and the mixture of them significantly decreased the severity of the disease at the rate of 43, 50.75 and 78.75 % respectively in comparison with controls. They concluded that application of *G. fasciculatum*, *P. fluorescens* and a mixture of the both on wheat decreased not only the severity of disease caused by *B. sorokiniana* but also increased the weights of both *Bipolaris* infected and non-infected plant.

In a survey, antagonistic effect of 280 strains of bacteria from wheat rhizosphere in Markazi province was studied on *B. sorokiniana*. The strains were identified as *Bacillus subtilis* (GI, GH2), *B. pumilus* (SH4, KH7), *Bacillus* sp. (H3), and *Pseudomonas fluorescens* bv. III (AS). In laboratory tests, all strains inhibited mycelial growth of *B. sorokiniana* isolates. All antagonists grew at 1, 10, 50 and 100 ppm of triadimenol and difenoconazole. In greenhouse experiments, selected antagonists were used as seed dressing and soil drenching. In seed dressing method, *B. subtilis* GH2 and *P. fluorescens* bv. III AS had the greatest effect on disease severity reduction and in increasing plant height, shoot and root dry weights, in the presence of the pathogen. In soil drenching method *B. subtilis* GH2 and *B. pumilus* KH7 showed better results (Mohammadi et al. 2005).

***Rhizoctonia* spp.**: Genus *Rhizoctonia* is one of the most important soil borne pathogens that invades different plants belonging to different families. *Rhizoctonia* bare patch is caused primarily by *R. solani* J. G. Kuhn, anastomosis group (AG) 8. Other AGs have been isolated from wheat roots, including AGs 2, 2-1, 4, 5, 9 and 10 but have low virulence or are of unknown pathogenicity (Paulitz 2010a). *R. solani* has been isolated from wheat roots in most parts of Iran (Foroutan et al. 1995, Arjmandian and Rohani 1998, Darvishnia et al. 1998, Safaee et al. 2000c).

Rhizoctonia cerealis causing sharp eyespot on wheat foot, in Iran, was first isolated from Mazndaran (Rahimian 1989) then Kermanshah province (Safaee 2000c).

Ravanlou *et al.* (2002) isolated some anastomosis groups of *Rhizoctonia* associated with wheat root and crown in Fars province in southern Iran. Then they distinguished three binucleate *Rhizoctonia* as AG-I, AG-G, AG-Bb and one multinucleate *R. solani* as AG-4 using standard testers. After pathogenicity test under greenhouse condition, only *R. solani* AG-4 caused brown discoloration and decay of root and crown of wheat.

The sensitivity of different field crops to isolates of *R. solani* was studied in order to apply more successful crop rotation program in Kerman province in south-east Iran. The results of disease severity evaluations showed that maize and wheat with disease severity indices (DSI) of 1.66 and 0.58 in laboratory condition and 1.46 and 2.63 in greenhouse had the minimum sensitivities respectively. Sugar beet and safflower with DSI 3/52 and 3/40 in laboratory condition and tomato and melon with 7/22 and 6/37 in greenhouse conditions were the most susceptible species to anastomosis groups 2, 3 and 4. Anastomosis group 4 had the highest disease while anastomosis group 3 had the lowest DSI under the two conditions of laboratory and greenhouse (Molaei et al. 2014).

Prospects

Based on the above review, in order to achieve a more effective management of FSBP, the following issues are suggested as research perspectives in Iran:

- Further investigations in order to reveal dominant species of FSBP in sub-region scales.
- Evaluating resistance of more wheat cultivars and genotypes against FSBP.
- Testing resistance of plants in Poaceae family other than wheat to find resistant sources for possible resistance transfer to wheat.
- Continuing research on mycorrhizae and plant growth promoting rhizobacteria for getting more effective isolates and combinations for biological control agents against each of FSBP or a group of FSBP.
- Improving formulations of biocontrol agents for the long-term sustainability.

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Determination of the *Fusarium* spp. relationship between wild grasses and wheat plants

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Summary

Besides many cultivated plants, wild grasses are also hosts of *Fusarium* species and those that are colonized may not exhibit disease symptoms. Wild grasses and wheat plants were taken during 2012, 2014, 2015 from a total of 136 location sites. Plant samples were identified at genus and species levels of *Aegilops, Avena, Bromus, Elymus, Festuca, Hordeum* and *Triticum*. A total 187 isolates of *Fusarium* spp. were identified from isolates obtained as a result of isolation studies while 17 species were determined morphologically at species level, fifteen isolates were identified as genus. Eleven of the species were also confirmed by species-specific primers. As a result of the survey, it was determined that the most common *Fusarium* species was *F. equiseti,* which was found in 23 of 53 locations, with an incidence of 43.4%. *F. culmorum*, which is considered to be the most important and common pathogenic species in our country among all *Fusarium* species, was found in 17 of the 53 locations with a rate of 32.1%, *F. culmorum* was the second most common *Fusarium* species, besides *F. poae* at nine, *F. graminearum* at four, and *F. avenaceum* at two locations in investigated wild grasses and wheat plants. *B. japonicus, B. hordeaceus, L. perenne, L. multiflorum, A. cylindrica, A. triuncialis, A.geniculata, Aegiops* sp., *Hordeum* sp were hosts of *F. culmorum. F. culmorum* has been found in both wheat plants and grasses at four locations .One of the important pathogens, *F. graminearum* was isolated from *Aegilops cylindrica, Aegilops* sp., *L. rigidum* and wheat plants from four locations. *F. poae* was found in wild grasses at six locations, while it was obtained from wheat fields at seven different locations. *F. oxysporum*was isolated from six grasses and 12 wheat plants locations in the study. The prevalence of *F. equiseti* in both wild grass and wheat plants suggests that more attention should be paid to this fungus in terms of both endophyte, pathogen or mycotoxins producers. When pathogenic *Fusarium* spp. were examined in terms of presence rates, it is understood that both wild grasses and wheat are found in close proportions. In particular, the presence of *F. culmorum* in both wild grasses and wheat in the same area strengthens the opinion that weeds may have a role in the spread of root rot and headblight diseases in wheat for our country.

Introduction

Multiple grass inhabiting fusaria are capable of causing seedling blights and root rots in crops, and if *Fusarium* communities change as a result of proximity to agricultural production, there may be an impact on natural host communities. (Zhang et al. 2016; Yang et al. 2018; Fulcher et al.2020). The Turkish grassland has multispecies communities and is an important source for feeding of livestock animals. Wild relatives of wheat in Türkiye show great diversity (Karagöz and Özberk 2010; Cabi et.al. 2010). The major grass species are grown in pasture are *Aegilops* spp., *Lolium* spp., *Hordeum* spp., *Elymus* spp., *Triticum* spp., *Secale* spp. and *Festuca* spp. in Türkiye (Davis et al. 1994). In North America and Europe, many *Fusarium* spp. have been isolated from many different cultivated cereal and wild grasses (Sprague 1950; Katan 1971; Jenkinson and Parry 1994; Inch and Gilbert 2003). Crown rot is most economically important in the bread and durum wheats, barley and triticale. However the host ranges includes many temperate cereals and grasses. It is therefore important to control grass weeds in rotations and fallows (Burgess et al. 2001).

According to study on wild grasses in Türkiye, *Fusarium equiseti* and *F. oxysporum* were isolated at high frequencies from all three agroecological regions. *Fusarium culmorum* was associated with half of the grass species examined, indicating that it is present in non-cropped grasslands as well as in cropping systems and it is speculated that *F. culmorum* is endemic in northern Türkiye. This is further supported by the isolation of *F. culmorum* from *Aegilops umbellulata*, which occurs predominantly in Türkiye (Bentley et al. 2006). However, no study has been found the relationship of *Fusarium* species seen in grass plants and wheat plants in the spread of the crown rot disease. The objective of this investigation was to compare wild grass plants and wheat for the presence of *Fusarium* spp. and wild grass hosts that may have important role in the survival and spread of pathogenic *Fusarium* spp.

Methods

Wild grasses plants were randomly collected from thirtheen provinces of central, east- western and northern Anatolia regions of Türkiye in 2012, 2014 and 2015 summer seasons (Figure 1). The grasses examined were collected from areas adjacent to commercial cereal crops. The plants, at 20 km from the selected routes, each one emphasizing a randomly collected wild grains encountered in the road to the right and left of 10 to 15 plants were collected, numbered, and brought to the laboratory in envelopes. By the end of June and the beginning of July 2012, 2014 and 2015, besides wild cereals, wheat plants were taken randomly and brought to the laboratory. Two pieces of crown parts randomly selected from five plants were surface sterilized in sodium hypochlorite (1% and then rinsed twice in sterile distilled water) and placed on tissue paper. Then, crown parts were plated on potato dextrose agar (PDA, Sigma) medium and two replicates were incubated under 24◦C, 12 h NUV+daylight/12 h dark conditions, in an incubator for a period of week. *Fusarium* spp. were transferredto synthetic nutrient agar (SNA) medium for identification. The diagnoses were made under the stereo-microscope and light microscope some of the fungi to the genus-level. Identification at the species level was carried out according to the identified criteria found principally in publications by Gerlach and Nirenberg, 1982 andSummerell et al., 2006. Identification was confirmed by PCR tests for 11 species with species-specific primers. Some *Fusarium* isolates that could not be identified were recorded at genus level (Table 1). On the other hand wild grasses were identified according to the diagnostic criteria of Davis, 1968 (Table 2).

Results

During the three-year surveys, *Fusarium* spp. was determined in 53 (39%) out of a total of 136 locations and one or more *Fusarium* isolates were obtained from these locations. A total of 187 *Fusarium* isolates were obtained and identified at the species level. As a result of the study, 17 *Fusarium* species were identified morphologically. In addition, eleven of the seventeen species were identified molecularly with species-specific primers (Table 1).

As a result of the survey study, it was determined that the most common *Fusarium* species was *F. equiseti* with a rate of 43.4% in 23 of 53 locations in wild grasses and wheat plantations. *F. culmorum*, among all *Fusarium* species, was found in 17 of the 53 locations which was the second common species, *F. poae* in nine locations, *F. graminearum* in four locations, *F. avenaceum* in two locations in investigated wild grasses and wheat plants. *F. culmorum* was obtained from wheat fields found in 9 locations and also found in 13 locations from wild grasses. It was found in both grown wheat and wild grasses of the four locations. *F. oxysporum*, which, is the third most common *Fusarium* species in wheat cultivation areas that was isolated from six locations in wild grasses and 12 locations in wheat plants. *F. graminearum* was isolated from *A. cylindrica, Aegilops* sp., and *L. rigidum* without any symptoms. *F. poae* was isolated from wild grasss at 7 locations and isolated from wheat plants at 6 locations. However, *F. poae* was isolated from only one of the same locations from wild *Hordeum* sp. and wheat plants. (Table 1). On the other hand, there is a difference in the distribution of *Fusarium* species between agro-ecological regions (Figure 1). For example, while *F. culmorum* was more isolated from the Thrace region, *F. equiseti* was most common in Middle East Anatolia and *F. proliferatum* in Midwest Anatolia. *F. tricinctum* and *F. inflexum* were detected only in Thrace region.

Discussion

Isolates of *F. equiseti* from *A. cylindrica, A. triuncialis, E. elongatus, Festuca* sp., *L. multiflorum* and *L. perenne* are a common colonizer of aging or dying plant tissues. It is also considered to be a primary colonizer of sorghum roots by Reed et al. 1983. This species produces group A and B trichothecenes as well as metabolites such as fusarochromanone, beauvericin and equisetin. *F. semitectum* was reported the most common species in Malaysia were studied by Nur Ain Izzati et al. (2009). It was most common in their study, while *F. equiseti* were found to be the most frequent species in our study. Different climatic conditions and the presence of different grass plants are thought to play a role in these differences.

F. equiseti, followed by *F. culmorum, F. oxysporum, F. acuminatum. F. equiseti* and *F. oxysporum* were isolated at high frequencies from all three agroecological regions (Figure 1). Burgess et al. (1981) found that the isolation of these species except *F. culmorum* from randomly selected healthy, diseased or senescing tissue is consistent with their classification as cosmopolitan saprobic *Fusarium* species. *F. culmorum* was associated with half of the grass species examined, indicating that it is present in non-cropping grasslands as well as in cropping systems, and it is speculated that *F. culmorum* is endemic in northern Türkiye. This is further supported by the isolation of *F. culmorum* from *Aegilops umbellulata*, which occurs predominantly in Türkiye (Bentley et al. 2006), and is the contributor of the common to U-genome all polyploid species in the section *Aegilops* (Kawahara 2002) including *A. tauschii* Coss., a progenitor of modern hexaploid bread wheat. These preliminary data are significant as wild species of the wheat group are useful sources of economically significant genetic material, such as disease resistance genes, for cultivated wheat breeding programs (Kawahara 2002). We found the isolation of *F. culmorum* from *Aegilops geniculata, Lolium perenne, Aegilops* sp., *Hordeum* sp., *Bromus hordeaceus, Bromus japonicus, Lolium multiflorum, Triticum aestivum* in northern Türkiye (Table 2).

F. oxysporum, which is not considered to be a pathogen of plants of the family Poaceae, was a species that was frequently isolated from the roots and lower internodes of the ornamental grasses. In this study, *F. oxysporum* and *F. solani* were found frequently on both grasses and wheat plants. In contrast, Szecsi et al. (2013) surveyed a large number of grasses but none of t he grass species had any *F. oxysporum* isolates. In the study, species such as *F. solani* and *F. oxysporum* were found in abundance in Minnesota prairie soils but were not found in the above-ground parts of grasses (LeBlanc et al. 2017). Tunali et al. (2006) has demonstrated *F. oxysporum, F. solani, F. subglutinans* and *F. acuminatum* isolates are generally weak pathogens of wheat, however, it was also determined that there were differences in virulence among the isolates. Kroon et al., (1991) also reported that pathogenic *F. oxysporum* species are further classified into opportunistic parasites, true pathogens and obligate pathogens, which are dependent on the specialization of fungal-plant interactions.

Isolations were made from the stem-bases of 1346 plants representing 15 weed species collected from three sites of fallow land; one following a crop of potatoes and two following wheat crops. Two hundred and twenty-six *Fusarium* isolates were obtained from 14 of the 15 weed species of which 114 (50·4%) were *F. avenaceum*, 88 (38.9%) were *F. culmorum*, 16 (7.1%) were *F. poae*, 6 (2.5%) were *F. sambucinum* and 3 (1.3%) were *F. graminearum*. No obvious symptom of *Fusarium* infection was observed on any of the plants sampled (Jenkinson and Parry, 1994). In our studies, it was determined that any symptoms were observed due to *Fusarium* spp. on the grasses.

There is a difference in the distribution of *Fusarium* species between agro-ecological regions (Figure 1). For example, *F. culmorum* was more isolated from the Thrace region, while *F. equiseti* and *F. proliferatum* were more isolated in midwest Anatolia. *F. tricictum* and *F. inflexum* were determined only in Thrace region. Some authors have proven that plant pathogens isolated from weeds are often more pathogenic compared to ones isolated from cultivated plants. Akinsanmi et al. (2007) determined that passage through alternative hosts changes the fitness of *F. graminearum* and *F. pseudograminearum*. They concluded that one passage through alternative hosts reduces pathogenicity, but at the same time provides better ability for colonisation of primary host. This could explain the lack of symptoms on alternative hosts. It is important to emphasize that their samples were surface sterilized and that weed did not exibit disease symptoms, which could mean that isolated *Fusarium* species live inside these plants as endophytes (Barrow et al. 2008). In another study, it has been reported that *F. graminearum* specis complex (FGSC) were found be present without showing symptoms in uninoculated flowers of *Elymus* plants in the glasshouse. FGSC strains were isolated from seed lots of *Elymus* and other native grass species (Chehri et al. 2011). In Manitoba Canada, wild grasses harbor several species of *Fusarium*, including *F. graminearum,* the predominant species causing fusarium head blight in cereal crops in Manitoba (Inch and Gilbert 2003). However, in our study. *F. graminearum* has been identified at only three locations in *A. cylindrica, Aegilops* sp., and *L. rigidum* (Table 2).

In conclusion, the prevalence of *F. equiseti* in both wild grass and wheat plants suggests that more attention should be paid to this fungus in terms of both endophyte, pathogen or mycotoxins producers. In addition, since *F. oxysporum* is frequently found in plants as an endophyte and can be used in biological control with its antagonist feature, its presence in both wild wheat and wheat plants is important in terms of control. In particular, the presence of *F. culmorum* in both wild grasses and wheat in the same area strengthens the opinion that weeds may have a role in the spread of root rot and head blight diseases in wheat for our country. These results showed that wild grasses are an important host for *Fusarium* species and have a partial role in the spread of cultivated plants. Correlation between wild grasses and cultivated cereal interactions on *Fusarium* spp. need future works.

Table 1. Percent of *Fusarium* spp. isolated from grass sampled in 2012, 2014 and 2015 years. Absence of the *Fusarium* species from wheat and grasses is indicated by a blank cell

***Fusarium* spp.**	**Grass**	**Wheat**	**Grass+Wheat**	**Same Locations**	**Incidence (%)**
F. equiseti	15	13	28	5	15.0
F. culmorum	11	10	21	4	11.2
F. oxysporum	6	12	18	1	9.6
F. solani	9	9	18	2	9.6
F. acuminatum	6	8	14	-	7.5
F. proliferatum	7	6	13	1	7.0
F. poae	6	7	13	4	7.0
F. semitectum	5	7	12	-	6.4
F. chlamydosporum	4	3	7	-	3.7
F. graminearum	3	1	4	-	2.1
F. redolens	1	2	3	-	1.6
F. tricinctum	-	3	3	-	1.6
F. verticillioides	3	3	-	-	1.6
F. subglutinans	2	1	3	-	1.6
F. inflexum	2	1	3	-	1.6
F. avenaceum	1	1	2	-	1.1
F. compactum	1	1	2	-	1.1
Fusarium sp.	13	7	20	?	10.7
Total	**92**	**95**	**187**	**17**	**100**

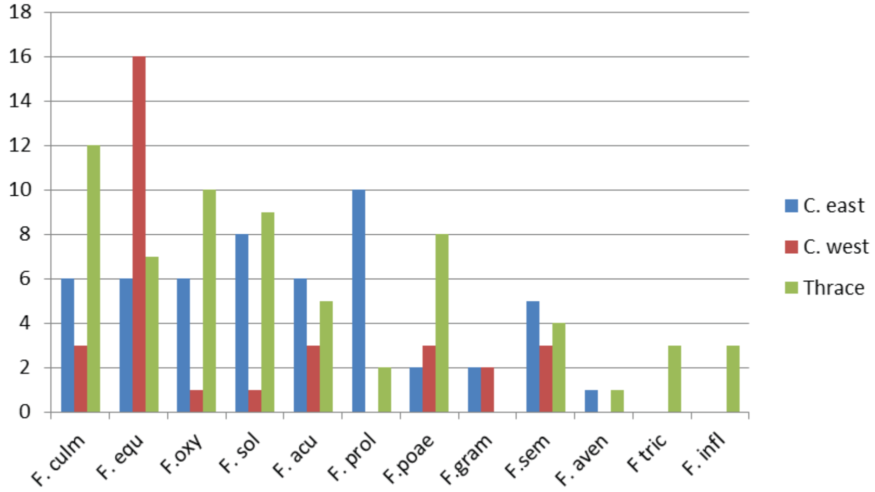

Figure 1. Frequency of isolation of the twelve *Fusarium* species from wheat and grass plants from three agro-ecological regions of Türkiye.

Table 2. Distribution of *Fusarium* species in wild grasses

Grasses	*Fusarium* species
Bromus japonicus	*F. acuminatum, F. oxysporum, F. culmorum*
Festuca sp.	*Fusarium sp., F. equiseti*
Hordeum bulbosum	*F. solani, F. inflexum, F. acuminatum*
Bromus hordeaceus	*F. culmorum, F. poae*
Lolium rigidum	*F. compactum, F. graminearum, F. solani, Fusarum* sp.
Lolium perenne	*F. culmorum, F. poae, F. equiseti, F. solani, Fusarium* sp.,
Lolium multiflorum	*F. chlamydosporum, F. culmorum, F. equiseti, F. solani, Fusarium* sp.
Aegilops cylindrica	*F. equiseti, F. culmorum, F. graminearum, F. sambucinum, F. armeniacum, Fusarium* sp., *F. tabacinum, F. subglutinans, F. solani, F. redolens, F. semitectum, F. proliferatum, F. chlamydosporum*
Aegilops triuncialis	*F. equiseti, F. acuminatum, F. poae, F. culmorum, F. oxysporum, F. semitectum*
Avena sativa	*F. inflexum, F. oxysporum, F. proliferatum, F. semitectum*
Bromus squarrosus	*F. acuminatum, F. subglutinans, F. solani, F. oxysporum, F. semitectum, Fusarium* spp.
Aegilops geniculata	*F. culmorum, F. semitectum, F. oxysporum, F. avenaceum, F. proliferatum*
Hordeum sp.	*F. culmorum, F. acuminatum, F. poae*
Aegiops sp.	*F. culmorum, F. proliferatum, Fusarium* sp.
Lolium sp.	*F. chlamydosporum, Fusarium* sp.
Elymus sp.	*Fusarium sp., F. acuminatum, F. poae*
Elymus elongatus	*F. chlamydosporum, F. oxysporum, F. equiseti*

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Current status of root and crown rot diseases of wheat and barley in Türkiye

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Summary

Wheat and barley are important crops in Turkish agriculture. There are numerous root and crown rot diseases of wheat and barley in the world. In this review, fungal pathogens causing root and crown rot of wheat and barley plants grown in Türkiye are presented. Although there are many pathogens reported causing root and crown rot diseases of wheat and barley in Türkiye, *Fusarium*, *Rhizoctonia*, and *Bipolaris* species seem to be more common.

Recent research related to root and crown rot diseases of wheat and barley in Türkiye

Wheat and barley are important crops in Turkish agriculture. There are numerous root and crown rot diseases of wheat and barley in the world (Bockus et al. 2010, Mathre 1982). Also in Türkiye, numerous root and crown rot diseases of wheat and barley are reported. Root and foot rots of wheat are often observed in wet areas with high precipitation, but in Central Anatolia, the disease can cause considerable damage in spite of limited rainfall. The results of the National Winter Cereals Research Project have revealed that *Helminthosporium* spp. and *Fusarium* spp. were the most prevalent agents of rot diseases and followed by *Gaeumannomyces graminis* var. *tritici* and *Pseudocercosporella herpotrichoides*. Highly resistant varieties were not available. The most affected varieties were Bezostaya, Bolal 2973, and Kunduru (Kınacı 1984).

Aktaş (1991) determined the reactions of some Turkish and German barley cultivars and lines to *Drechslera sorokiniana*. Barley lines and cultivars G76-H-86, KABVD2017, KABVD2023, YEA-734-2, Eshter, Sigra, Doris, Tapir, Mahmut, Corona, Adonia, Gerbel, Catinka, Optima, and Detto showed moderately resistant reactions to root inoculation.

Muratçavuşoğlu and Hancıoğlu (1995) conducted a survey to determine *Fusarium* species causing root and crown rot of wheat in Ankara province, Türkiye. Thirty-one isolates of *Fusarium* species were obtained. Using the soil inoculation method, 15 isolates were found as pathogenic to wheat cultivar Gerek 79. Two isolates of *Fusarium culmorum*, 8 isolates of *F. acuminatum*, 4 isolates of *F. graminearum*, and 1 isolate of *F. heterosporum* were pathogenic.

Aktaş et al. (1996) carried out a study in the Sakarya province of Türkiye to determine wheat root and crown rots. It has been found that root and crown rot diseases were common in this area. As the causal agents of wheat root and crown rots, *Fusarium culmorum*, *F. graminearum*, *Rhizoctonia cerealis*, *Drechslera sorokiniana*, *Alternaria alternata*, *F. moniliforme*, *F. equiseti*, *Acremonium kiliense*, *Ophiobolus graminis*, *Pseudocercosporella herpotrichoides*, *Pythium graminicola*, *Phoma* spp., and *Stemphylium herbarum* were determined. Among these, *F. culmorum*, *Rhizoctonia cerealis*, *Drechslera sorokiniana*, *F. moniliforme*, *Pythium graminicola*, *Ophiobolus graminis*, and *Pseudocercosporella herpotrichoides* were found as the most important ones in that region.

Aktaş *et al.* (1997) carried out a study under field conditions. *Fusarium culmorum*, *Rhizoctonia cerealis*, *Drechslera sorokiniana*, *Ophiobolus graminis*, *Fusarium moniliforme* and *Pythium graminicolum* determined at the experiment field of Sakarya Maize Research Institute were also used during the reaction studies, carried out under the controlled conditions. Zitnika, P. Niska, Zamber, GK-Szrjke, and Kinesrj were found Resistant (R), S. Zaitna, Slavonis, Sivka, Nada-Pozezanka-Zitarka, Lonja, Rona-2, Imerio, Partizanka, Balkan, Marmara-86, Sagvari, Kare A-1, Öthalom, GK-32-82, Mömtchil, MV-12(Alt.-12), GK-cize and Centomion wheat varieties and lines were found Moderately Resistant (MR) during the reaction studies carried out under natural conditions.

Mamluk *et al.* (1997) conducted extensive field surveys of wheat and barley diseases in the Central Anatolian Plateau of Türkiye. The most frequently occurring disease of the wheat was foot and root rot and *Fusarium* spp. and *Microdochium nivale* were the predominant pathogens isolated. In addition to these fungi, *Cochliobolus sativus* and *Wojnowicia graminis* were also isolated from diseased wheat plants. In barley, the most frequently occurring disease was barley leaf stripe, followed by foot and root rot. *Cochliobolus sativus* was the main pathogen causing foot and root rot. In addition to *C. sativus*, *Microdochium nivale*, *Fusarium* spp., and *Gaeumannomyces graminis* were also isolated from diseased barley plants.

Demirci (1998) obtained 98 isolates of *Rhizoctonia* spp. from wheat and barley grown in Erzurum, Türkiye. Of these, 78% were *R. solani* (AG-2 type 1, AG-3, AG-4, AG-5, and AG-11), 10% were binucleate *Rhizoctonia* (AG-I and AG-K) and the remainder were *Waitea circinata* var. *circinata (Rhizoctonia* sp.). Among the binucleate *Rhizoctonia*, AG-I was not recovered from barley. In pathogenicity tests on wheat and barley, the highest disease severity was caused by isolates of AG-4 and AG-11, whereas isolates of AG-2 type 1, AG-3, AG-5, and *W. c.* var. *circinata* were moderately virulent. Isolates of binucleate *Rhizoctonia* were all nonpathogenic.

Eken and Demirci (1998) determined *Drechslera sorokiniana* incidence on sub-crown internode and crowns of barley and wheat samples collected from Erzurum, Türkiye. Under the field conditions, the mean percentages of disease ratios were found at 46.8 % in wheat, 51.1 % in barley in 1994; 48.9% in wheat, and 54.3% in barley in 1995. *D. sorokiniana* was the most pathogenic to cv. Hawk and the least pathogenic to cv. Lancer in the greenhouse.

In Konya province, Türkiye, 200 fields from different locations were investigated for root and foot rot diseases of winter cereals (Aktaş et al. 1999). The mean disease intensity was 36.21%. Cereal root and foot rots caused 5-9% grain yield losses. From the samples taken, 29 different fungi were identified. The responses of cereal varieties to *Drechslera sorokiniana* (*Cochliobolus sativus*), *Ophiobolus graminis* (*Gaeumannomyces graminis*), *Fusarium culmorum*, *F. moniliforme* (*Gibberella fujikuroi*) and *Rhizoctonia cerealis*, in terms of spikes/m2, 1000-kernel weight and mean grain yield were determined. Thirty varieties and/or promising lines were tested under greenhouse conditions. Barley cultivars Erginel 90 and Kıral 97; triticale cultivar Tatlıcak 97 and advanced triticale line BDMT 19 were resistant to all tested pathogens. Six chemicals at two application rates were tested for pathogen control. The best results were obtained with Premis Universal at 250 ml/100 kg seeds and Divident 2DS at 300 g/100 kg seeds.

Aktaş et al. (2000) examined 218 barley and wheat fields of Eskişehir province of Türkiye for root and crown rots and 194 of them were found to be infested. Twenty-four fungi species of 8 genera among the samples collected from the infested fields were determined. Fourteen out of these 24 species were *Fusarium* spp., 3 were *Drechslera* spp., 2 were *Alternaria* spp., 1 from each of *Ophiobolus graminis*, *Nigrospora oryzae*, *Ulocladium atrum*, *Phoma* spp. and sterile fungus. One-hundred-ninety-nine barley varieties and lines chosen from the cross-bred field of Anatolia Agricultural Research Institute were used in barley seed pathology, together with 99 wheat varieties and lines. *Rhizopus stolonifer*, *Alternaria alternata*, *A. triticina*, *Septonema* spp., *Cladosporium herbarum*, *Penicillium* spp., *Ulocladium atrum*, *Fusarium moniliforme*, *Aspergillus niger*, *A. parasiticus*, *Alternaria* spp., *Phoma* spp., *Stemphylium herbarum*, *Drechslera teres*, *D. tetramera*, *Curvularia inaequalis*, *Mucor* spp., *D. sorokiniana*, *A. candidus*, *Trichoderma viride*, *Lacellina* spp., *Ustilago hordei* and sterile fungi were isolated from the barley seeds. Reactions of 199 barley and 99 wheat varieties and lines to *D. sorokiniana* were determined. A wheat variety and 36 barley varieties and lines were found to be Resistant (R), and 39 barley and 10 wheat varieties and lines were found to be Mid Resistant (MR) to *D. sorokiniana*.

Bağcı et al. (2001) conducted a study in 1999-2001 to determine the genetic sources for root rot tolerance and the effects of root rot pathogens on yield in cereals in two different experiments in Konya, Türkiye. In both experiments, clean and inoculated seeds were used. The inoculum used was prepared by using *Drechslera sorokiniana, Fusarium culmorum*, and *Fusarium avenaceum* which had been reported in earlier studies as widespread pathogens in the region. For the determination of resistance-tolerance sources, 1329 genotypes of wheat, barley, and triticale originating from national and international programs were used. Of 1329 entries, 269 were found to be tolerant. The ratio of tolerant genotypes in national germplasm was 44% while 15% in international germplasm. In international germplasm, the ratios of tolerant genotypes were 23% and 7%, developed for rainfed and irrigated areas, respectively. In yield trials, 10 genotypes (5 bread wheat, 2 durum wheat, 2 barley, and 1 triticale) were tested. On average, root rot pathogens caused a 34% yield reduction. The highest yield reduction, 54%, occurred in durum wheat cultivar Kızıltan-91 while the lowest, 13%, was observed in triticale cultivar Tatlıcak-97. In terms of tolerance, the species had the order of triticale> barley>bread wheat>durum wheat from the highest to the lowest.

Demirci (2003) determined the resistance status of 10 wheat cultivars grown in Türkiye to *Fusarium graminearum*, *F. culmorum*, and *Bipolaris sorokiniana*. Disease severity and emergence values were recorded. Cultivars Bezostaja 1 and Gün 91 were moderately resistant to *F. culmorum*. Cultivars Bezostaja 1, Kutluk, Kırgız 95, Gün 91 and Dağdaş 94 were moderately resistant to *B. sorokiniana*. *F. graminearum* caused high disease severity in all cultivars. Only cv Mızrak showed a moderately susceptible reaction to *F. graminearum*.

Demirci and Dane (2003) obtained 468 *Fusarium* and *Fusarium*-like isolates from crowns and sub-crown internodes of winter wheat grown in Erzurum, Türkiye. Of these isolates, 34.8% were *Fusarium acuminatum*, 32.3% were *Fusarium equiseti*, 16.9% were *Fusarium oxysporum*, and 15.0% were *Microdochium nivale*, 0.6% were *Fusarium tabacinum* and 0.4% were *Fusarium solani*. In pathogenicity tests on wheat, the highest disease severity was caused by isolates of *M. nivale*, whereas isolates of *F. oxysporum*, *F. acuminatum*, *F.*

equiseti and *F. solani* were slightly virulent; isolates of *F. tabacinum* were nonpathogenic.

CIMMYT in collaboration with the Turkish Ministry of Agriculture and Rural Affairs have established an International field and laboratory screening program for identifying winter and spring wheat accessions with resistance to *Fusarium culmorum* and *F. pseudograminearum* and *Bipolaris sorokinana*. There were a number of spring and winter wheats that provided a high level of resistance better than the known check varieties (2-49 and Sunco). Major progress has been made with the spring wheat material, where a number of crosses (Sunco/Pastor) provided a higher level of resistance and yields. Materials have been specifically bred to develop multiple disease resistances, such as GS50AT34/Sunco which combines crown rot with root lesion nematode. Some of the preliminary yield data from the winter wheats indicated that some of the materials with a high level of resistance, for example, the released Turkish variety Dağdaş 94, also had a high level of tolerance, which is equivalent to the more tolerant triticale, Tatlıcak 97 and barley, Erginel 91. Another widely grown variety Gerek 79, offered some resistance, but unfortunately no tolerance (Nicol et al. 2004).

Hekimhan et al. (2004) investigated the tolerance of 20 winter cereals to the dryland root rot complex (*F. culmorum*, *F. pseudograminearum*, and *B. sorokiniana*) for 3 consecutive years through inoculated field trials in Çumra, Türkiye. The data clearly indicated that significant yield loss occurred. The degree of intolerance was greatest with durum wheat > bread wheat > triticale > barley.

Mert-Türk et al. (2004) investigated the antifungal activity of oat root extract against soil-born cereal fungal pathogens. The growth of fungal colonies was observed until control plates covered the medium. The researchers found that the reaction of fungi to the extract varied greatly not only among the species but also against different concentrations, too. Compared to control plates, there was no difference in the growth rate of *Pythium ultimum*. However, the growth of *Rhizoctonia solani*, *Curvularia* sp., and *Fusarium nivale* was restricted to 10%-30% of the control plates. *F. culmorum*, *F. avenaceum*, *F. poae*, and *F. graminearum* also exhibited reduced growth rates which varied between 30%-60% depending on the species.

Uçkun and Yıldız (2004) investigated the pathogens causing root and crown rot diseases in wheat areas of Denizli, İzmir, and Aydın provinces of Türkiye. The incidence and intensity of the diseases were 58.28% and 25.07%, respectively. The most commonly isolated fungi from root and crown were *Fusarium* spp., *Dreschlera sorokiniana, Rhizoctonia cerealis* and *Alternaria alternata*. The pathogenicity range of *R. cerealis* and *Fusarium* spp. isolates showed differences among 26% to 76% and 0% to 72%, respectively. The most pathogenic species were found to be *F. culmorum* and *R. cerealis.*

Bentley et al. (2006) conducted a survey of *Fusarium* species associated with wheat stem bases in northern Türkiye. The *Fusarium* species associated with stem bases of 6 grass species were also studied. From wheat stem bases, fifteen *Fusarium* species were isolated. Three of these species and 2 additional species were isolated from the grasses. The species isolated included the important pathogens *F. culmorum* and *F. pseudograminearum*, as well as a number of saprobic species and secondary colonizers. *Fusarium culmorum* was the most prevalent pathogenic species, and isolated from 28% of sites surveyed. Another pathogenic species, *F. pseudograminearum* was isolated from 8% of sites.

Tunali et al. (2006) obtained 51 *Fusarium* isolates from crown tissues of winter wheat plants in the Central Anatolian Plateau of Türkiye. The 51 isolates which represented 13 different species were assessed for *Fusarium* pathogenicity using the wheat cv. Pehlivan. Virulence ratings were variable with *F. culmorum, F. pseudograminearum,* and *F. graminearum* causing the greatest severity and reduced plant weight. Other species including *F. oxysporum, F. subglutinans, F. acuminatum, F. equiseti, F. verticilloides* and *F. solani* were weak pathogens on cv. Pehlivan and under these conditions would not be considered pathogens.

Erginbaş et al. (2007) screened some actinomycetes isolates from soil against wheat root rot fungi. The effectiveness of *Streptomyces* species against *Fusarium culmorum, F. graminearum, F. moniliforme,* and *B. sorokiniana* ranged between 44-71%. *Streptomyces* isolate 129.01 inhibited all root rot fungi tested effectively. Under *in vitro* conditions *Fusarium culmorum, F. graminearum, F. moniliforme,* and *B. sorokiniana* were inhibited by this isolate at 62, 63, 35, and 68%, respectively.

Hekimhan et al. (2007a) investigated the effect of seeding rate on dryland root and foot rot disease in selected winter bread wheat varieties. The results indicated that disease inoculation had a highly significant effect on both grain yield and disease intensity. There were significant differences between varieties for disease intensity. The researchers found some differences from year to year in seed rate and disease intensity and yield. However, there was no clear relationship (2 or 3 way) over the years between seeding rate, disease intensity, and/or grain yield. Hekimhan et al. (2007b) investigated the effect of some fungicides for bunt and smut diseases on root and foot rot diseases in Selçuklu-97 and Seri-82 wheat varieties. The seeds were inoculated with *Bipolaris sorokiniana, Fusarium culmorum,* and *F. pseudograminearum* two days before sowing, and the fungicide applications were done one day before planting. Statistical differences were determined for grain yield, fungicide application, disease intensity, application rate, and the effect of fungicides on disease intensity. The averages for grain yield were 3.84 tons/ha for Seri -82 and 3.02 tons/ha for Selcuklu-97, and the combined average was 3.43 tons/ha. Disease intensity was 55% for Selcuklu-97 and 50% for Seri-82. Averaged over both varieties was 56% for 150 g fungicide rate and 49% for 200 g rate. The effectiveness of fungicides was ranged as difenoconazole (36%>carboxin (33% > triticonazole (31% > diniconazole (20%).

Tunalı et al. (2007) investigated the use of *Trichoderma* spp. for biological control of *Fusarium* species causing crown rot of wheat. Fifteen isolates of *Fusarium* spp. were selected and potting soil was inoculated. On the other hand, wheat seeds were treated with *Trichoderma* spp. including *T. puliliferum*, *T. atroviride*, and 2 different isolates of *T. harzianum* or Maxim XL 035 FS and Lamardor 400 FS fungicides. Seeds treated with fungicides and biological control agents were sown in pots containing sterile soils or soils inoculated with each disease agent individually. At the end of the trials, plants with their roots were taken from the pots and individually evaluated. Statistically significant differences were found among the application of biological control agents and fungicide, pathogen inoculation, and interaction between them. Statistical analysis revealed that while plants inoculated with *Fusarium semitectum* 41 and *F. pseudograminearum* 40 showed the highest disease rate, *F. equiseti* 23-2-1 was the species causing the least disease. Duncan test applied for results including biological agents, fungicides, and control blocks showed that Lamardor 400 FS fungicide constituting the group C was the most effective suppressor of the disease agents followed by Maxim XL 035 FS fungicide (BC). Biological control agents *T. puliliferum* ranked third after fungicides followed by *T. atroviride*, however, isolates of *T. harzianum* were in the same group as the controls.

Akgül (2008) examined root, and crown rot diseases of winter wheat and the possible *Fusarium* species in wheat fields of the Çukurova Region, Türkiye. In addition, the effects of different fungicide treatments, fertilization practices, and cultivar reactions were investigated against Fusarium foot rot disease development caused by *F. culmorum*. All of the examined fields were found to be infested with the disease. *F. culmorum, F. oxysporum, F. equiseti, F. verticilloides* and *F. semitectum* were isolated from infected plants. *Fusarium* was the predominant genus among the fungal genera. No stable and considerable tolerance was observed in the 12 different wheat cultivars against the disease. In the pot experiments, disease development decreased when calcium ammonium nitrate (CAN, 26% N) or combined fertilizer (15.15.15 %, NPK) were preferred to 20.20.0 % NPK. Disease severity was lower with ammonium nitrate (33% N) fertilized plants than that of urea (46% N) fertilized ones. The efficacy of seed treatment with tebuconazole reached 47.8% and it was the most effective fungicide. When plants were sprayed twice with fluquinconazole, tebuconazole, and epoxyconazole + carbendazim, the disease severity was reduced by 96.3, 93.9, and 91% respectively. In the field experiment, no statistical difference has been found in fungicidal seed treatments but the combination in which the plants that fertilized CAN plus ammonium nitrate and sprayed with tebuconazole gave the most successful results.

Tunali et al. (2008) determined the fungi associated with wheat crowns and roots in cereal-producing areas of Türkiye. More than 26% of the fields had one or more of the fungal species commonly reported as part of the dryland root rot complex, *F. culmorum* (14%) > *B. sorokiniana* (10%) > *F. pseudograminearum* (2%). The fungi considered to be part of the high rainfall root rot complex were found at very low frequencies: 3% for *Pythium* spp. and 2% for *Gaeumannomyces graminis*. *Rhizoctonia* species were found in 22% of the fields. Several *Fusarium* species considered to be less or nonpathogenic to cereals were also found in high frequencies at 11% (*F. oxysporum*, *F. chlamydosporum*), 10% (*F. sporotrichioides*), and 8% (*F. avenaceum* and *F. solani*). The authors reported that crown rot caused by *Fusarium culmorum* was the dominant causal species in Türkiye.

A nursery of 121 diverse spring and winter wheat lines with susceptible and resistant checks for crown rot (CR) were screened under inoculated field and greenhouse conditions for two consecutive years. Twenty-eight lines were promising and were further evaluated in both fields and in the greenhouse. The variables assessed both in the greenhouse (root and crown score) and field (white head development and crown score) were found to be correlated, and the variety effect was significant for these variables. Out of the 28 lines assessed over multiple years, two originating from the joint Türkiye-CIMMYT-ICARDA International Winter Wheat Improvement Program and two spring wheat lines from Australia were identified to provide resistance equivalent to or better than the best known published resistance sources, 2-49, and Sunco. These 4 wheat lines may be useful in breeding for resistance against CR (Erginbaş et al. 2009).

Erkan and Yılmaz (2009) determined *Polymyxa graminis* ribotypes in wheat production areas in Samsun province of Türkiye. Wheat (Kutluk 94) and barley (Tokak) plants were grown in these soil samples using the bait plant technique. *Polymyxa graminis*, the vector of soilborne wheat mosaic virus, barley mild mosaic virus, wheat spindle streak mosaic virus, and barley yellow mosaic virus, was analyzed using stained root samples. *P. graminis* was found in 4.8% (6 samples) of wheat samples and 3.2% (4 samples) of barley samples. According to host range studies, ribotypes of *P. graminis* were determined using soil samples containing each of ten *P. graminis* isolates. Ten different plant species (wheat, barley, rye, corn, oat, triticale, pearl millet, rice, poa, sorghum) were grown in these soils and maintained at 13-19°C. *P. graminis* cysts (resting spores) were visualized in the roots of these plants by staining with acid fuchsin and using microscopy. Ribotype I (temperata), of which host is known to be only barley, was identified in the Havza district while other isolates were found to be ribotype II (tepida) in samples collected from the Bafra, Alaçam, and Kavak districts, which infects wheat as well as other cereals. Besides this, it was determined that *P. graminis* isolates found in the roots of wheat can infect corn, oat, barley, triticale, pearl millet, poa, and sorghum.

With the aim of determining root and foot-rot diseases, a study was carried out on 18 various cultivars of wheat in 4 districts of Sakarya province of Türkiye (Araz et al. 2009). *F. culmorum*, *Fusarium graminearum*, *F. crookwellense*, *F. subglutinans*, *F. moniliforme*, *F. oxysporum*, *F. solani*, *F. dimerum*, *F. equiseti*, *F. sporotrichoides*, *F. acuminatum*, *Rhizoctonia* spp. and *Alternaria* spp. were identified from the samples collected. *F. graminearum* was obtained from 10 wheat cultivars and *F. culmorum* was obtained from 5 wheat cultivars.

Arıcı and Koç (2010) surveyed *Fusarium* sp. affecting wheat crops in Adana, Türkiye. The isolates were identified as *F. graminearum, F. culmorum, F. croockwellense* and *F. avenecaum*. *Fusarium graminearum* was the predominant pathogen isolated, followed by *F. culmorum, F. avenecaum,* and *F. croockwellense. F. graminearum* and *F. culmorum* isolates showed genetic variation.

Hekimhan (2010) carried out a study in the Thrace region of Türkiye in order to determine the root and crown rot diseases of wheat and the factors affecting the pathogenicity of the causal agents of fungi. Disease prevalence (100%) and disease severity (32%) values were 100% and 32%, respectively. Pathogens causing diseases were determined as *Fusarium* sp., *Rhizoctonia* sp., *Pseudocercosporella* sp., *Cochliobolus* sp., *Cephalosporium* sp., *Rhizopus* sp., *Pythium* sp., *Gaeumannomyces* sp., and *Alternaria* sp. *Fusarium culmorum* was the most common pathogen. The reaction of 63 wheat varieties to *F. culmorum* was determined. With the application of acibenzolar-S-methyl, a 21.81% reduction in pathogenicity was achieved by promoting resistance, but phytotoxic effects were also observed. By applying plant nutrient tonic, the pathogenicity of *F. culmorum* decreased by 13.25% under field conditions and by 11.25% under greenhouse conditions compared to control. On the other hand, the effect of *F. oxysporum* was found to be insignificant in the study conducted on the pathogenicity of *F. culmorum* through the promotion of resistance by the application of saprophyte *F. oxysporum*.

In soil, seed, and seedling inoculation of *F. culmorum*, disease severity was determined at the same level in soil (35.49%) and seed (34.02%) inoculation, while it was determined at a lower level (27.96%) in seedling inoculation.

Gürel et al. (2010) identified genetic diversity in *F. culmorum* isolates collected from wheat fields in Türkiye. One-hundred and four molecular markers were evaluated and similarity comparisons were shown as a dendrogram. According to the Rep-PCR data, the average genetic diversity was 52.3% ranging from 15.8% to 88.7%. Cluster analysis showed agreement with the distance of sampling locations. The highest genetic similarity was determined between two *F. culmorum* isolates that originated from the same agro-ecological region.

Hekimhan et al. (2011) reported that *Hymenula cerealis* (syn. *Cephalosporium gramineum*) caused disease in wheat plants in the East Mediterranean Region of Türkiye.

Ölmez et al. (2011) conducted surveys on wheat at Muş, Bitlis, and Van provinces of Türkiye to determine root and crown rot prevalence and severity. Disease prevalences were 77% and 73.7% at Muş, 71.4% and 68.5% at Bitlis, 69% and 63.2% at Van provinces in 2009 and 2010, respectively. Disease densities were 12.5% and 11.2% at Muş, 14.2% and 12.6% at Bitlis, 8.4% and 7.1% at Van provinces in 2009 and 2010, respectively. From diseased plants, *Fusarium, Rhizoctonia, Bipolaris, Curvularia, Pythium, Epicoccum, Alternaria, Penicillium, Torula,* and *Rhizopus* species were isolated. Seventy-eight *Fusarium* and *Bipolaris* isolates were identified to species level. The most common species were *Bipolaris spicifera* (17%) and *Fusarium acuminatum* (16.5%).

Çetin et al. (2011) determined the prevalence of *Pythium* spp. in wheat-growing areas of Söke, South Marmara, Thrace, and Adana regions of Türkiye. Pocket diagnostic kits were used to determine *Pythium* spp. presence in the samples. In Söke 83.3%, In South Marmara 75%, and in Thrace 50%, and in Adana 16.7% of samples showed positive reaction and therefore infected with *Pythium* spp.

Ünal and Dolar (2012) conducted a survey in the Eskişehir and Ankara provinces of Türkiye for determining anastomosis groups and pathogenicity of *Rhizoctonia* species associated with wheat root and crown rot. Pathogenicity tests showed that *Rhizoctonia solani* AG 8 caused the common symptoms of damping-off and stunting.

Çepni et al. (2013) carried out a study related to the molecular characterization of *Fusarium* spp. collected from cereal fields in Türkiye. The selection of restriction enzymes for IGS-RFLP studies has been found critical to maximizing polymorphic markers. Three of 14 restriction endonucleases were useful in differentiating *Fusarium* spp. isolates. PstI was the most efficient enzyme to produce a maximum of nine DNA markers in one individual and a total of 22 polymorphic representative banding patterns. Polymorphism based on IGS-RFLP was high. There was no association between IGS diversity and geographic locations from which the samples were taken. Both MAT-1 and MAT-2 sequences were amplified in *F. graminearum.* Most of the *F. culmorum* isolates carried either MAT-1 or MAT-2 sequences, and two isolates carried both sequences. Mating type determination was helpful to distinguish *F. pseudograminearum* from *F. graminearum.*

Ünal et al. (2013) surveyed wheat fields in the Ankara, Konya, Yozgat, Eskişehir, and Kırıkkale provinces of Türkiye in order to identify *Rhizoctonia* species. Three *Rhizoctonia* isolates collected from necrotic lesions on the crown and root were identified as *Rhizoctonia zeae* (teleomorph: *Waitea circinata* var. *zeae*), as well as four of the isolates from rhizosphere soils. Pathogenicity tests showed that *R. zeae* caused a significant reduction of emergence, stunting, reduction in the number of seminal roots, and superficial discoloration on the hypocotyls and roots.

Ünal et al. (2014) conducted a study to identify the anastomosis groups and pathogenicity of binucleate *Rhizoctonia* species from soil samples in wheat production areas of Ankara, Konya, Eskişehir, Yozgat, Kayseri, Kırıkkale, Nevşehir, Kırşehir, and Aksaray provinces of Türkiye. Fifty-one isolates were identified as binucleate *Rhizoctonia*.These isolates were found to belong to AG A, AG C, AG D (*R. cerealis*), AG E, AG G, AG H, AG I and AG K. The groups other than AG D were not found to be pathogenic on susceptible wheat cultivar.

Surveys were conducted in Kırşehir, Kayseri, Aksaray and Nevşehir provinces of Türkiye for the determination of the pathogenicity, anastomosis groups, and species of *Rhizoctonia* in wheat production areas (Ünal et al.

2015a). A total of 53 *Rhizoctonia* isolates were obtained. Forty-five of these isolates were from the plants, and 8 of them were from the soil. The multinucleate isolates belonged to *Rhizoctonia solani* AG 3, AG 4 HG II, AG 5, and *Waitea circinata* var. *circinata* anastomosis groups. Binucleate *Rhizoctonia* isolates were AG I and AG K. Pathogenicity tests revealed that AG 4 HG II and *Waitea circinata* var. *circinata* groups were pathogenic on wheat, and the most virulent group was found to be AG 4 HG II.

Ünal et al. (2015b) determined the reactions of some wheat cultivars against *Rhizoctonia cerealis* AG D, *Rhizoctonia solani* AG 4, AG 5, AG 8, *Waitea circinata* var. *zeae* and *Waitea circinata* var. *circinata.* Cultivar Demir 2000 and cv. Ankara 98 were the most susceptible cultivars showing susceptiblity against all AG groups. Cultivar Cemre was the most resistant, which was also found as moderately susceptible against AG D, AG 8, and *W. circinata*. var. *circinata* and moderately resistant against *W. circinata.* var. *zeae* and AG 5. All cultivars tested in this study showed a susceptible reaction against AG 4 which was the most virulent group.

Fusarium redolens (Gebremariam et al. 2015) and *Fusarium hostae* (Gebremariam et al. 2016) have been reported as the causal agents of crown rot on wheat (*Triticum* spp.) in Türkiye.

Gebremariam et al. (2018) determined the diversity and pathogenicity of *Fusarium* species associated with crown rot of wheat in Türkiye. The isolates were identified as *F. culmorum, F. graminearum, F. pseudograminearum, F. acuminatum, F. equiseti, F. hostae, F. brachygibbosum, F. redolens, F. oxysporum, F. avenaceum, F. proliferatum, F. torulosum, F. flocciferum, F. incarnatum, F. solani, F. tricinctum,* and *F. reticulatum*. *Fusarium equiseti* was the most commonly isolated species. Among the damaging species, *F. culmorum* was the most predominant species being isolated from 13.29% of sites surveyed while *F. pseudograminearum* and *F. graminearum* were isolated from limited areas. All the 342 isolates belonging to the 17 *Fusarium* species were tested for pathogenicity on susceptible durum wheat cultivar Kızıltan-91. Seven out of the 17 *Fusarium* species tested for their pathogenicity caused crown rot in different levels of severity. *Fusarium culmorum*, *F. graminearum* and *F. pseudograminearum* caused severe crown rot disease in cv. Kızıltan-91. *Fusarium hostae* and *F. avenaceum* were moderately pathogenic. *Fusarium redolens* and *F. acuminatum* were weakly pathogenic. On the other hand, *F. equiseti*, *F. oxysporum*, *F. solani*, *F. reticulatum, F. incarnatum*, *F. tricinctum, F. flocciferum, F. torulosum, F. brachygibbosum,* and *F. proliferatum* were non-pathogenic. *Fusarium culmorum* isolates differed in their aggressiveness on the susceptible durum wheat cv. Kızıltan-91. The most aggressive *Fusarium culmorum* isolate Fc2 was used as inoculum to screen 165 spring wheat lines for their reaction (Gebremariam et al. 2020). Thirteen percent of the lines tested showed promising and consistently resistant/moderately resistant reactions to *Fusarium culmorum*.

In order to determine root rot diseases surveys were carried out in wheat and barley fields of Kırıkkale and Kırşehir provinces of Türkiye (Yeğin 2015). From diseased plants *Rhizoctonia solani* AG3, *Rhizoctonia solani* AG4, *Waitea cir.* var. *circinata*, binucleate AG 1, *Fusarium oxysporum*, *F. chlamydosporum, F. acuminatum, F. redolens, F. equiseti, F. incarnatum, Microdochium nivale, F. tritinctum, Ophiosphaerella herpotricha, Bipolaris sorokiniana, Alternaria alternata, Curvularia inaequalis, Embellisia* spp., and *Phaeosphaeria pontiformis* were isolated. Among the isolates, for wheat, *M. nivale,* and *F. oxysporum* and for barley, *F. oxysporum* were found as the most common, respectively. As a result of pathogenicity tests *M. nivale, Waitea cir.* var. *circinata*, *F. acuminatum, F. oxysporum, F. tritinctum* on wheat and *M. nivale, R. solani* AG4, *F. acuminatum, F. oxysporum, F. redolens, F. chlamydosporum, B. sorokiniana* on barley were found to be pathogenic.

Under greenhouse conditions, Hekimhan et al. (2016) conducted a study to determine the resistance of 39 barley varieties against *Fusarium culmorum* and *F. graminearum* (group 1). A higher number of varieties showed resistance to *F. graminearum* group 1 than the *F. culmorum*. Ten varieties tested were found to be resistant to both fungi.

Erginbas-Orakci et al. (2016) assessed three different inoculation techniques (colonized grain, seedling dip, and stem base droplet) using two pathogenic isolates of *Fusarium pseudograminearum* and *F. culmorum*. Disease severity was greater in seedling dip, compared to colonized grain, and stem base inoculation, respectively. The colonized grain method produced an adequate level of severity and consistent cultivar ranking in both experiments. Results showed significant inoculation method x cultivar interactions.

Erginbas-Orakci et al. (2018a) identified novel QTL linked to crown rot resistance in spring wheat. Three marker-trait associations (MTAs) were identified linked to crown rot resistance. Two of these MTAs on chromosome 3B were associated with field crown scores, each explaining 11.4% of the phenotypic variation, and the third MTA on chromosome 2D was associated with greenhouse stem score and explained 11.6% of the phenotypic variation.

Erginbas-Orakci et al. (2018b) screened 141 wheat genotypes and breeding lines for their resistance reactions to an isolate of *F. culmorum* under growth room, greenhouse, and field conditions in Türkiye. Seventeen genotypes were found as moderately resistant at the adult growth and/or seedling stage. The genotypes from Mexico seemed to have adult plant resistance rather than seedling resistance which was higher in the USA genotypes. Winter bread wheats PATWIN YR5 and TAST/SPRW//ZAR/5/YUANDONG 3/4/PPB868/CHRC/3/PYN//TAM101/AMIGO which possess a high level of resistance seemed promising for breeding for foot rot.

Fusarium pseudograminearum is an important *Fusarium* species that causes root, crown, and foot rots in wheat. Under both growth room and greenhouse conditions, Yılmaz (2018) evaluated 199 durum wheat genotypes and 5 control genotypes to *Fusarium pseudograminearum*. Under growth room conditions, 15, 20, 134, and 30 genotypes exhibited resistant, moderately resistant, moderately susceptible, and susceptible reactions, respectively. Under greenhouse conditions; 19, 16, 121, and 43 genotypes were found resistant, moderately resistant, moderately susceptible, and susceptible, respectively. Two durum wheat genotypes showed seedling and adult plant resistance to *F. pseudograminearum*.

Köycü and Özer (2019) obtained 40 *Fusarium* spp. from the root and foot parts of wheat in the Trakya Region of Türkiye. Disease severity values of *Fusarium* spp. ranged between 0% to 100%. Isolate 14 (*Fusarium culmorum*) exhibited the highest infection in Flamura-85 and Pehlivan cultivars and it was the most pathogenic isolate. Six isolates showing disease severity of >40% on these cultivars were selected for resistance tests. Pre-emergence damping-off and disease severity in the seedling stage were at the lowest in the cultivars of Gelibolu and Esperia, respectively.

Ölmez and Tunalı (2019) determined 19 different *Fusarium* species in wheat production areas of Diyarbakır, Şanlıurfa, Mardin and Adıyaman provinces of Türkiye. *F. proliferatum* was the most frequently isolated species (17.4%). Important root and crown rot pathogens *F. culmorum* and *F. pseudograminearum* constituted 13% of the isolates.

Başer et al. (2021) investigated the effects of sowing time, genotype, and seed fungicide pre-treatments on root and crown rot and grain yield in bread wheat. The effect of different seed fungicide applications on root and crown rot of bread wheat cultivars was found to be statistically significant. The highest root and crown rot was determined for the earliest sowing and the lowest root and crown rot was obtained from the second sowing time. Among the four different fungicide pre-treatments, the highest root and crown rot was obtained in the control application. The lowest root and crown rot was obtained in prothioconazole + tebuconazole, carboxin + thiram, and prochloraz + triticonazole, with 1.96%, 2.10%, and 2.19% root and crown rot, respectively.

Ahmadi et al. (2022) investigated the interactions among *Fusarium culmorum*, *Heterodera filipjevi*, and drought on a set of wheat germplasm with differing levels of resistance/ tolerance to *H. filipjevi*, *F. culmorum*, and drought. Plant emergence was significantly reduced when seeds were planted in a water stress environment where *H. filipjevi* and *F. culmorum* were present. Crown rot was more severe under water stress compared with *Fusarium* inoculation alone. The number of cysts fell significantly when *H. filipjevi* was co-inoculated with *F. culmorum*. Yield components were significantly reduced by each of the single stress factors and losses were greater when plants were exposed to double or triple stress factors.

Sohail et al. (2022) genotyped 189 advanced spring bread wheat lines with 4056 SNP markers and screened for *Pratylenchus* spp. and *Fusarium culmorum* resistance. Results indicated that the genotypes could be divided into five subpopulations. Genome-Wide Association Studies were carried out for both resistances to *Pratylenchus* and *Fusarium* species. Eleven different SNPs on chromosomes 1A, 1B, 2A, 3A, 4A, 5B, and 5D were significantly associated with root-lesion nematode resistance. Seven markers demonstrated association with *Pratylenchus neglectus*, and four markers were linked to *Pratylenchus thornei* resistance. Eight markers on chromosomes 1A, 2B, 3A, 4B, 5B, and 7D were associated with *Fusarium* crown rot resistance.

Under growth room conditions, Yazıcı et al. (2022) evaluated the seedling resistance reaction of 200 bread wheat genotypes plus 6 control genotypes obtained from CIMMYT to *Fusarium pseudograminearum*. Out of the 200 tested genotypes; 1 (0.5%), 35 (17.5%), 112 (56%), 45 (22.5%), and 7 (3.5%) were resistant, moderately resistant, moderately susceptible, susceptible and very susceptible to *Fusarium pseudograminearum*, respectively.

Conclusion

It appears that there is a wide variety of fungi causing root and crown rots of wheat and barley plants. However, *Fusarium, Rhizoctonia,* and *Bipolaris* species seem to be more common and important. Research should focus on important pathogens and control methods should be developed.

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Associations of novel candidate genes markers with resistance to *Bipolaris sorokiniana* – wheat common root rot disease

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Summary

Common root and crown rot (CRR), *Bipolaris sorokiniana* (Sacc.) is one of the important diseases in wheat, causing considerable yield losses globally. Resistant sources are a feasible and effective method for management of plant disease . However, the knowledge on mechanisms of resistance is scarce. We screened 33 wheat genotypes against *B. sorokiniana* under greenhouse and field conditions. In addition, real-time quantitative PCR (qPCR) analysis using ten novel candidate gene markers, *Cre3*, *EDS1*, *LTP5*, *PGIP, PR-1*, *PIEP1, TLP*, *UGT*, *Stb6*, and *PFT* were conducted on leaf and root. Lowest disease severity was on 'Alvand', followed by 'Baharan' and Bam as resistant genotypes respectively. Quantitative gene expression showed that, although the candidate defense genes were up-regulated ranging from 1.24 to 3.5-fold in wheat roots and leaves inoculated with *B. sorokiniana*, they were highly regulated in resistant 'Alvand', 'Mehregan' and 'Bam'. *Cre3*, a resistance gene to cereal cyst nematodes, *Heterodera filipjevi* was regulated in cultivars resistant to *B. sorokiniana*. Similar results were obtained with *Stb6* gene resistant to *Septoria tritici* blotch, *EDS1* to powdery mildew, *Blumeria graminis*, and *PR-1* and *UGT* genes to leaf rust, *Puccinia triticina*. Conclusively, *T. aestivum - B. sorokiniana* interaction was using defense-related genes in wheat protection for sustainable development. However, further studies shed a new light on simultaneous resistance to other diseases in wheat cultivars.

Introduction

Crown and root rot disease (CRR) is an important disease that damages wheat and its products. CRR is caused by a soil-borne fungal pathogen, *Bipolaris sorokiniana* (Sacc.) Shoemaker (Pleosporaceae) that is common in some tropical and temperate regions (Zolfaghary et al. 2021). Seedling necrosis, brown spots on the leaves, root rot, and black point are important crop limiting factors in hot and humid regions in the world (Al-Sadi 2021; Arabi et al. 2019). The rate of damage and decreased crop due to this disease vary in different regions, but 35% losses in wheat yield has been estimated in 12% of the total wheat cultivation in the Pacific Northwest of the United States (Al-Sadi 2021; Li et al. 2021).

Expression of *PR-1* and *PR-5* (a stress-responsive multifunctional pathogenesis-related family), and other genes is known to be associated with the salicylic acid (SA) and jasmonic acid (JA) signaling pathway (Kaur et al. 2021; Moatamedi et al. 2018). Despite the importance of PR genes in providing disease resistance, the role of these genes in wheat resistance against *B. sorokiniana* is not well studied (Li *et al.* 2021; Tehrani *et al.* 2020). However, the overexpression of PR genes has potential to confer a certain degree of resistance to fungal infections in many plant species (Kaur et al. 2021; Li et al. 2017).

Therefore, considering the importance of finding a control measure to combat this devastating disease, 33 universal wheat genotypes were screened for resistance to *B. sorokiniana* in the field and greenhouse to identify novel resistant sources. However, the findings regarding local (roots) molecular defense responses compared with systemic (leaves) defenses before and after *B. sorokiniana* inoculation in wheat is scarce. To our knowledge, this is the first study to investigate changes in the activity of novel candidate gene expression patterns in contrasting wheat cultivars upon soil inoculation with *B. sorokiniana*, and also to provide a valuable resource for future functional genomics studies to understand the simultaneous resistance to other diseases in wheat cultivars.

Methods

Thirty-three wheat genotypes, including domestic, exotic cultivars, and inbreeding lines were provided by the Seed Institute, and Plant Improvement Karaj, Iran. The genotypes were screened previously against *B. sorokiniana* under both greenhouse and field conditions as described by Arabi et al. (2019) and Ghazvini et al. (2018), located at the Agricultural and Natural Resource Research and Education Center, Isfahan, Iran, during 2015–2016 and 2016–2017 growing seasons. Of these, three resistant 'Bam', 'Mehregan', 'Alvand', and three susceptible 'Heidari' 'Karkheh', and 'Behrang' wheat genotypes were selected, and re-inoculated as above with *B. sorokiniana* for further studies in this research.

Total RNA was isolated using BioFACT™ Total RNA Prep Kit (Biofact, Korea), in accordance with the manufacturer's instructions. RNA concentration and purity were assessed using a Nanodrop NP80 spectrophotometer (Implen Germany). The cDNA was synthesized from 5 μg of total RNA using the cDNA Synthesis commercial kit (BioFACT™ 2X RT Pre-Mix) according to the manufacturer's instructions (Bagheri et al. 2020; Tehrani et al. 2020). Gene-specific primers were designed using Oligo7 software on *Triticum aestivum* L. gene sequences at the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov).

The primers used in the qRT-PCR analyses are shown in Fig. 1. The specificity of designed primers was confirmed by performing the PCR reaction on synthesized cDNA and genomic DNA (to confirm the accuracy of the PCR) (Moghaddam *et al.* 2019). The mRNA expression levels of ten candidate gene markers (*Cre3*, *EDS1*, *LTP5*, *PGIP*, *PR-1*, *PIEP1*, *TLP*, *UGT*, *Stb6*, *PFT* and *ACT* as a control) were analyzed by quantitative real-time PCR (qRT-PCR) using SYBR Green PCR Master Mix (2X) (Biofact, South Korea) according to the manufacturer's protocols. The qRT-PCR using StepOne™ 48-well Real-Time PCR System (Applied Biosystems) was performed with the thermal profile 95 °C for 15 min, 45 cycles of 95 °C for 15 s, annealing temperature depending on the primer Tm for 25 s and 72 °C for 40 s Fig. 1) (Moatamedi et al. 2018). The experiment was performed with five biological replicates for each sample and two technical replicates. LinRegPCR program (version 2012.3) were used to determine Primer efficiencies (E) for all primers and Ct values, and relative gene expression ratio computed based on Pfaffl method (Pfaffl 2001). The EF1-α gene (accession number NM_001288491.1) was used as the internal control to normalize the data (Bagheri et al. 2020; Moghaddam et al. 2019; Wei et al. 2018).

Result

To confirm the results obtained from the greenhouse and field experiments, the resistant 'Bam', 'Mehregan' and 'Alvand' as well as the susceptible 'Karkheh', 'Heidari' and Behrang' were re-screened three times in the greenhouse. Highest disease severity in the three respective experiments was in 'Karkheh' with 100, 85 and 85% (mean= 90%), followed by 'Roshan-Beck-Cross', 'Shabrang' and 'Heidari' with 80, 77 and 77 %, respectively. The lowest disease severity was in 'Alvand' and 'm909', with an average of 23 and 14%, respectively (P = 0.01) (Table 1).

Variance analysis of transcriptional changes in *Cre3* showed a significant increase in infected leaf and root tissues of resistant cultivars in all three times after soil inoculation. The highest increase in *Cre3* gene expression was in the second time (2 wpi), in 'Alvand', 'Mehregan' and 'Bam' with 2.06, 1.24 and 1.44 fold higher than the control, respectively. There was significant positive correlation between relative *Cre3* expression level in roots and leaves (r =0.781**) (Fig. 1A). *EDS1* expression was in both tissues in 'Alvand' with 1.56, 1.89 and 1.78-fold at wpi after inoculation in leaves, and 1.54, 1.79 and 1.63-fold in the roots, respectively. There was significant positive correlation. *EDS1* expression was in both tissues in 'Alvand' with 1.56, 1.89 and 1.78-fold at 1, 2 and 3 wpi after inoculation in leaves, and 1.54, 1.79 and 1.63-fold in roots, respectively. Also, significant positive correlations were recorded for relative *EDS1* expression level in leaves and roots (r =0.812**) (Fig. 1B). Highest increase in *LTP5* gene transcription was in 'Alvand' on 2 wpi with 2.17-fold in leaves and 2.91-fold in roots. There

Table 1. Mean comparison of the disease severity as influenced by the individual effects of six wheat varieties and their interaction to *Bipolaris sorokiniana*

S/No Variety		Means of Disease severity (%)				F value	P value	Reaction
		Test 1	Test 2	Test 3				
1	Karkheh	$^{\dagger}100^{a} \pm {}^{\Psi}0.0$	$^{\dagger}85^{a} \pm 2.9$	$^{\dagger}85^{a} \pm 2.9$	$^{\dagger\dagger}90^{A} \pm 2.8$	16.1^{**}	<0.0001	Susceptible
2	Heidari	$75^{b} \pm 2.9$	$75^{b} \pm 2.8$	$80^{ab} \pm 0.0$	$77^{B} \pm 1.4$	1.79	0.1758	Susceptible
3	Behrang	$49.3^{gh} \pm 2.5$	$54.6^{ce} \pm 2.7$	$52.0^{CD} \pm 2.0$	$51.9^{c} \pm 2.2$	0.002	14.5^{**}	Susceptible
4	Bam	$15^{d} \pm 2.9$	$33^{c} \pm 1.7$	$10^{c} \pm 0.0$	$20^{d} \pm 3.4$	27.7^{**}	<0.0001	Resistant
5	Mehregan	$25^{c} \pm 2.9$	$18^{de} \pm 1.2$	$15^{c} \pm 2.9$	$19^{d} \pm 1.9$	5.66^{**}	0.0056	Resistant
6	Alvand	$15^{d} \pm 2.9$	$13^{ef} \pm 1.7$	$10^{c} \pm 3.4$	$13^{e} \pm 1.2$	1.36	0.2644	Resistant
Test mean		$^{\dagger\dagger}45^{A} \pm 5.9$	$42^{B} \pm 5.7$	$40^{C} \pm 6.3$	C.V% = 8.9			
F value		237.5^{**}	225.6^{**}	276.8^{**}				
P value		<0.0001	<0.0001	<0.0001				

†Means having the same lowercase letter in each column (comparing wheat varieties in each test) and the ††means having same uppercase letter in right column (comparing individual effect of wheat variety) and below row (comparing individual effect of test) are not significant difference according to LSD ($p<0.05$), *and ** Significant at $p<0.05$ and $p<0.01$, respectively. S: Susceptible, R: Resistant. $^{\Psi}$Standard Error of Mean (SEM). †Means having same letters in each column are not significant difference according to LSD ($p<0.01$). $^{\Psi}$Standard Error of Mean (SEM).

was significant positive correlations between expression levels of this gene in both leaves and root tissues (r = 0.791 **) (Fig. 1C). Among resistant cultivars, 'Alvand' showed the highest increase in expression level of *PGIP1* gene, with 2.25, 3.03 and 3.31-fold in leaves at three time points, and 1.94, 2.92 and 2.47-fold in root tissue, respectively. Moreover, significant positive correlations were recorded for relative *PGIP1* expression level in leaves and roots (r =0.688 **) (Fig. not shown). We observed an increase in the activity of the *PR-1* gene in roots and leaves with a maximum expression level at 2 wpi. The highest increase in expression was in 'Alvand', and the least change in expression level was in Karkheh. Significant positive correlations were recorded for relative *PR-1* expression level in leaves and root (r =0.876 **) (Fig. not shown). Variance analysis of transcriptional changes in *PIEP1* and *TLP* gene showed that treatment, tissue, time after soil inoculation and cultivar and the interaction of these factors had a significant effect on changes in expression levels of these genes (P<0.01). The *PIEP1* and *TLP* were up-regulated in infected leaves and roots of all cultivars in the three time point (Figs. not shown).

Variance analysis of transcriptional changes in *UGT* gene in 'Alvand' showed the highest increase in leaf and root tissue, with 1.66, 2.22 and 2.01-fold in leaves at three time points and 1.74, 1.82 and 1.65-fold in root tissue, respectively. Increase in gene expression level in root tissue of 'Heydari' was significant only at 2 wpi. *Stb6* transcripts were up-regulated in infected roots and leaves of resistant cultivars at three time points after soil inoculation. Whereas, a very low level expression of *Stb6* was detected in susceptible cultivars compared to controls (Fig. 1). 'Mehregan' had the highest increase in expression at 1 wpi, 2 and 3 wpi. Whereas, 'Alvand' and 'Bam' showed a gradual increase in infected leaves that reached the highest expression level at 3 wpi, with 2.19-fold in 'Alvand' and 2.09-fold in 'Bam' compared to the controls. The correlation between the expression levels of this gene in both leaves and root tissues in 'Alvand' and 'Heydari' was significantly positive (r = 0.845 **). Transcriptional changes in *PFT* gene had a significant upward trend compared to controls, and reached its maximum at 3 wpi. Also, significant positive correlations were recorded for relative *PFT* expression level in leaves and roots (r =0.775 **).

Discussion

Resistant cultivars can be a useful tool in reducing the disease below threshold levels (Qostal et al. 2019). Genetic resistance is not only having environmental impacts, but also is widely applicable and economically feasible to reduce pesticide application. In this study, reaction analysis of wheat genotypes to CRR disease, *B. sorokiniana*, showed that the lowest CRR disease severity was on 'Alvand', 'Baharan', 'm90-9' and 'Bam' as the resistant wheat genotypes. Similar trials by Samiei et al *(*2008) on a few Iranian wheat genotypes to CRR disease indicated that wheat genotypes react differentially supporting our results except the 'Alvand' genotype. In Samiei *et al (*2008) the 'Alvand' genotype was considered as moderately susceptible, whereas in our trails, 'Alvand' genotype was introduced as a resistant one. The reasons might be due to the use of fungal isolates of *B. sorokiniana* that may cause variable disease levels.

Furthermore, these genes exhibited different levels of transcript accumulation in investigated cultivars and the higher levels of expression of these genes was observed in 'Alvand' and 'Bam'. All these results strongly indicated that under *B. sorokiniana* stress conditions root and leaf tissues of different wheat cultivars employed the same mechanisms to cope with *B. sorokiniana*. In addition to the significant variations in resistance, the expression pattern of ten candidate defense-related gene markers regulated significantly at a higher level in both tissues of resistant wheat cultivars upon inoculation with *B. sorokiniana*.

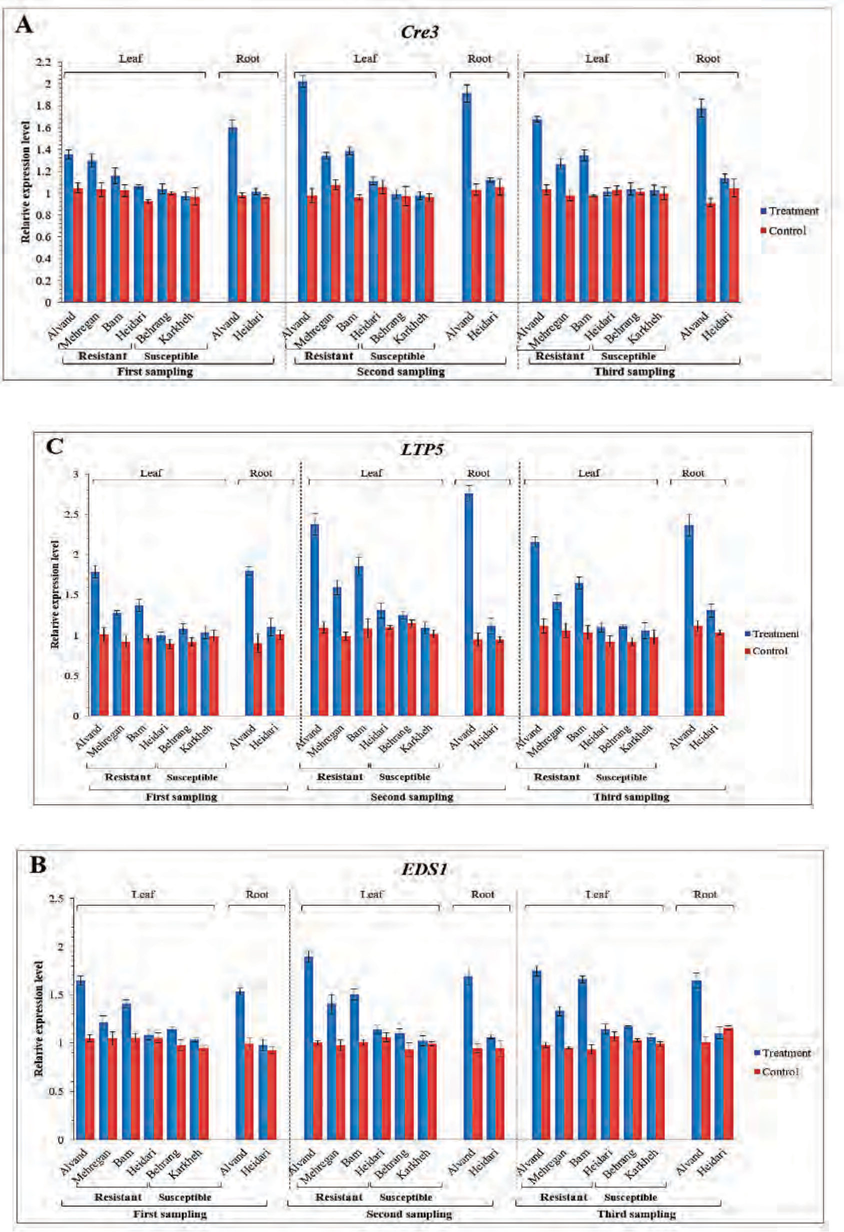

Figure 1. Transcript levels of *Cre3*, *EDS1* and *LTP5*, as samples in the leaf and root at three points after soil inoculation of three resistant genotypes, 'Alvand', 'Bam' and 'Mehregan' and three susceptible ones,'Karkheh', ''Heydari'' and 'Behrang'. Data represent the means ± SD (n = 5) of five biological replicates. Different letters above the bars denote significant differences at P <0.05.

Although genes and defense responses involved in the wheat- *B. sorokiniana* remain unexplored, numerous studies suggest the vital role of TF families such as PR-1 and PR-5 (TLP) in regulating defense genes like PR genes, integrate hormone and other signals producing an increased resistance against pathogen infection. In the current study, a significant accumulation of *Cre3* transcripts was induced in resistant wheat cultivars after infection with *B. sorokiniana*, indicating constitutive expression of *Cre3* enhances resistance against *B. sorokiniana* too. Our result is in excellent accordance with previous findings, which demonstrated the association of *Cre3* in resistance response against *Heterodera filipjevi* (Moatamedi et al. 2018). It is therefore not surprising that *Cre3* in wheat might have a similar role during wheat - *B. sorokiniana* interaction.

Up until now, few studies reported on defensive response mechanism of *PR-1* (pathogenesis-related protein 1) and *UGT* (UDP-glycosyltransferase) TFs against *B. sorokiniana* in wheat, and there are still many unknowns. Therefore, we selected *PR-1* and *UGT* as candidates and our results demonstrate that they are enhanced by *B. sorokiniana* in resistant cultivars 'Alvand'. Previous studies showed that wheat *PR-1* and *UGT* genes, are strongly induced under CRR, *B. sorokiniana* and leaf rust, *Puccinia triticina*, the major fungal diseases in wheat (Cui et al. 2021; Tianqing et al. 2018). Our results indicated that stable expression of *PR-1* and *UGT* increased resistance against both CRR and rust diseases, supported by Cui et al (2021). However, further investigations are needed to clarify the role of *PR-1* and *UGT* genes in wheat signaling in response to *CRR* and leaf rust.

Three *EDS1* genes were cloned from common wheat and were demonstrated to positively regulate resistance to powdery mildew, *Blumeria graminis* f. sp. *tritici*, the most common foliar disease of wheat, but the possible function of this gene in *T. aestivum* - *B. sorokiniana i*nteraction is still unknown. Here, we report on common wheat orthologous genes of *EDS1* family and their function in CRR resistance to *B. sorokiniana*. Even though this interaction is not the same, we found a similar profile in our experiment, in which *EDS1* showed activation in resistant wheat cultivars to *B. sorokiniana*. The *EDS1* proteins play important roles in plant basal resistance and TIR-NB-LRR protein-triggered resistance in dicots (Chen *et al.* 2018). Similar to *EDS1* gene, the possible function of *TPL, LTP5, PFT, PIEP1* and *PGIP1* genes against *B. sorokiniana* regulated in resistant 'Alvand' with the highest levels in *PGIP1*, followed by *PIEP1* and LTP, indicating they could thrive well in response to *B. sorokiniana* infection.

Stb6 transcripts were also up-regulated in infected wheat cultivars resistant to *B. sorokiniana*. It was highest in 'Mehregan', whereas 'Alvand' and 'Bam' showed a gradual increase in infected leaves that reached the highest expression level at 3 wpi, supported by Chartrain et al (2005) and Li et al (2021). Our results indicate *Stb6* holding simultaneous resistance to both the CRR, *B. sorokiniana* and *Septoria tritici* blotch diseases, in consistency with Chartrain et al. (2005) to *Septoria tritici* blotch, controlled by *Stb6* only. Consistent with our reports, a set of primary hexaploid synthetic wheat under high disease pressure, powdery mildew, leaf and stem rust at Omsk, Moscow suggests presence of new genes and diverse types of resistance (Shamanin et al. 2019).

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Fusarium spp. associated with crown and root rot in Azerbaijan and Kazakhstan

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Summary

Comprehensive surveys were conducted to identify *Fusarium* species associated with crown and root rot of wheat throughout the main wheat-growing areas of Azerbaijan and Kazakhstan in 2017 and 2019, respectively. Samples were taken from affected plants' roots and stem bases from 76 and 65 fields for Azerbaijan and Kazakhstan, respectively. The identification was conducted with morphological and molecular tools such as species-specific PCR and DNA sequencing of the internal transcribed spacer (ITS) and translation elongation factor 1- alpha (*EF1-α*) loci. Among *Fusarium* spp., *Fusarium culmorum* was found in the greatest number of fields with 192 isolates, followed by *F. acuminatum,* and *F. equiseti, F. pseudograminearum, F. graminearum*, *F. incarnatum*, *F. avenaceum*, *F. hostae*, *F. oxysporum*, *F. proliferatum*, *F. algeriense*, and *F. brachygibbosum* for Azerbaijan. At the same time, *F. acuminatum* was present in 66.15% of the fields surveyed and the most pre-dominant fungal species isolated for Kazakhstan, followed by *F. equiseti*, *F. culmorum*, *F. oxysporum*, *F. redolens*, *F. pseudograminearum*, *F. flocciferum*, *F.* cf. *incarnatum*, *Fusarium* sp., and *F. torulosum*. Azerbaijan is the second country after Algeria in which *F. algeriense* was detected. The findings of this investigation demonstrate the presence of a diverse spectrum of *Fusarium* species relevant to wheat crown and root rot in Azerbaijan and Kazakhstan.

Introduction

Wheat (*Triticum* spp.) is a primary source of calories and protein (Shiferaw et al. 2013), grown on 219 million ha and yielding 760.9 million tons (FAOSTAT 2022). Bread wheat (*Triticum aestivum* L.) and durum wheat (*T. durum* Desf.) provide a major contribution to the diets of humans and livestock in Azerbaijan and Kazakhstan, with an average annual production of about 1.82 million tons in a planted area of about 0.59 million ha and with 14.3 million tons produced on 12.1 million ha, respectively. Especially, Kazakhstan's wheat yield (1182.5 ton/ha) falls far short of the global average (3474.4 ton/ha) due to biotic and abiotic stressors.

A variety of root and stem base rot complex diseases frequently hinder wheat yields, stands, and grain quality in wheat-growing countries (Kumar et al. 2002, Kazan and Gardiner 2018, Su et al. 2021). The complex fungal species attacking the crown and root tissues of wheat causes a serious problem, including damping-off, blight, necrosis, and dry rotting of the root, crown, sub-crown, and lower stem tissues, along with wilting and stunting of seedlings and mature wheat plants (Bockus et al. 2010), resulting in significant yield reductions in the major wheat-producing regions of the world (Gonzalez and Trevathan 2000). Although most dominant species in this complex can change yearly in wheat production areas, several *Fusarium* species have been reported in the species complex: *Fusarium pseudograminearum* O'Donnell & T. Aoki (teleomorph: *Gibberella coronicola* T. Aoki & O'Donnell), *F. graminearum* Schwabe (teleomorph: *G. zeae* (Schwein.) Petch), *F. culmorum* (Wm. G. Sm.) Sacc., *F. avenaceum* (Fr.) Sacc. (teleomorph: *G. avenacea* R. J. Cook), *F. equiseti* (Corda) Sacc. (teleomorph: *G. intricans* Wollenw.), *F. oxysporum* Schltdl., *F. acuminatum* Ellis & Everh. (teleomorph: *G. acuminata* Wollenw.), *Microdochium nivale* (Fr.) Samuels & I. C. Hallett (syn. *F. nivale* (Fr.) Sorauer), and *F. algeriense* I. Laraba & K. O'Donnell (Cook and Veseth 1991; Gonzalez and Trevathan 2000; Hill et al. 1983; Laraba et al. 2017; Moya-Elizondo et al. 2011; Paulitz et al. 2002; Smiley and Patterson 1996; Smiley et al. 2005; Tunali et al. 2008). These fungi have complex population dynamics and interact with one another in crop residues, soil, and seed to survive between wheat crops. Multiple fungal species frequently co-occur at the same time and may interact in synergistic or competing ways, influencing their development and disease-causing capabilities (Grudzinska-Sterno et al. 2016). To combat the disease, a thorough understanding of pathogen biology and populations, as well as the epidemiological factors that contribute to disease exacerbation, is required. The first step in this process is to collect data on the distribution and prevalence of wheat pathogens associated with this phenomenon, especially since these data could be beneficial in predicting future changes in disease distribution patterns.

Fusarium crown rot (FCR) is caused by numerous *Fusarium* spp.; however, several surveys conducted to investigate the species causing FCR have revealed that the most common and devastating species are *F. culmorum* and *F. pseudograminearum* in many wheat-growing regions of the world (Backhouse et al. 2004; Cook 1981; Moya-Elizondo et al. 2011; Shikur Gebremariam et al. 2018; Smiley and Patterson 1996; Tunali et al. 2008). Although *F. culmorum* prefers lower temperatures than *F. pseudograminearum*, summer temperatures in the dryland area increase the prevalence of both pathogens (Cook 2010; Dyer et al. 2009). These species have been frequently isolated from infected plants but a complex etiology of FCR has been demonstrated by several studies (Backhouse et al. 2004; Fedel-Moen and Harris 1987; Laraba et al. 2017; Smiley and Patterson 1996).

Little is known about the distribution and prevalence of pathogens of underground parts of winter wheat in Azerbaijan and Kazakhstan. In this study, a detailed survey study was conducted to assess the distribution of pathogen the country to generate information and understand disease dynamics, as well as to test the pathogenicity of the obtained species on a susceptible wheat cultivar.

Methods

Extensive surveys were conducted to collect samples showing typical symptoms of crown and root rot from 76 wheat fields in the main wheat-growing regions of Azerbaijan in 2017 and 65 wheat fields in 2019 at the crop's maturity stage and harvesting time. The surveyed fields were arbitrarily chosen, with a distance of 5–15 km apart. From an area of 5-ha, 20–30 symptomatic wheat plants were randomly sampled in a zig-zag pattern, with each plant being 20 m apart and placed in paper bags. Samples were brought to the laboratory and kept at 4 °C until fungal isolation.

Collected plants were cut approximately 25 cm above the base of the stem. Before examining the stems for lesions, the crown, root, and stem base tissues were washed thoroughly under tap water for 15 min to remove soil particles. Small portions of necrotic and discolored crown, sub-crown, and root tissues of sampled plants were superficially disinfested for 1 min with 1% sodium hypochlorite solution, rinsed with sterile water, and blotted dry. The dried sections were chopped into 1 cm lengths and placed on 1/5 strength potato dextrose agar (PDA; diced potato tubers (40 g) were cooked in water for 15 min, the strained broth was brought to 1 L, and dextrose (4 g) and agar (15 g) were added and then autoclaved) fortified with streptomycin (0.1 g/L) and chloramphenicol (0.05 g/L) to inhibit bacterial contamination. Fungal colonies isolated from the sections were sub-cultured on new full-strength PDA (200 g diced potato tubers, 20 g dextrose, and 15 g agar) plates and purified using the hyphal tip or the single spore isolation method after five days of incubation in the dark at 23 °C. Fusarium-like colonies were also transferred to Spezieller Nährstoffarmer Agar (SNA) medium (1 g KH_2PO_4, 1 g KNO3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 dextrose, 0.2 sucrose, 20 g agar, distilled water to 1 L) and incubated at 23 °C for 10 days to enhance conidia and chlamydospore production (Leslie and Summerell 2016).

Isolation frequency and field incidence of species were estimated after species identification of each isolate. Isolation frequency was determined by dividing the number of fungal isolates per species by the total number of isolates obtained and expressing the value as a percentage. The incidence of individual species in the fields was calculated by dividing the number of locations from which fungal species were retrieved by the total number of fields surveyed and expressing the value as a percentage.

Template DNA was extracted from 50–100 mg of fungal powder using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The translation elongation factor 1- alpha (EF1-α) gene and the internal transcribed spacer (ITS) region of ribosomal DNA were amplified with EF1/EF2 (O'Donnell et al. 1998) and ITS1/ITS4 (White et al. 1990) primer sets, respectively. The PCR products were bi-directionally sequenced by Macrogen Inc. (Seoul, Korea) with the same primers. The DNA sequences were edited, and consensus sequences were computed manually using Mega X: Molecular Evolutionary Genetics Analysis across computing platforms (Kumar et al. 2018). All sequences were compared against the GenBank database, National Center for Biotechnological Information, using the BLASTn algorithm, and representative isolates for each species were deposited in GenBank.

Following species identification, representative isolates of identified species were chosen from various locations to ensure geographic representation and pathogenicity testing on wheat seedlings under growth room conditions of 12/12 h light/dark regime and at 23 °C. Common bread wheat (*Triticum aestivum* L.) cv. Seri 82 seeds were sterilized for 2 min in 1% NaOCl and then placed in plates containing a piece of sterile filter paper saturated with water to enhance germination. Plastic pots (15 cm diameter and 17 cm height) were filled with a potting mixture of peat (KTS 1, Klasmann-Deilmann, Germany), sterile vermiculite, and sterile soil (1:1:1, v/v/v). Five identical seedlings were placed on the soil surface of each pot. For inoculation of *Fusarium* spp. isolates, mycelial plugs (9 mm diameter) were cut from the margin of a growing PDA culture of representative isolates and placed onto the mixture substrate in the pots; then, the seedlings and the mycelial plugs were covered with the same potting mixture (Özer et al. 2020). A sterile agar plug was used as a control treatment. Each seedling was placed on a mycelial plug and covered with the mixture substrate. Five seeds were planted in each pot, and three replicated pots were employed for each isolate. All experiments were conducted twice. Plants were evaluated six weeks after incubation at a 12 h photoperiod at 23 °C. The index system with a 1–5 scale of Nicol et al. (2001) was used to assess the disease symptoms. Disease severity scores obtained from the pathogenicity were analyzed for significance by analysis of variance, followed by Fisher's least significant difference test at P = 0.05, with the Statistical Analysis System (SAS version 9.0; SAS Institute Inc.; Cary, NC, U.S.A.).

Results and discussion

In total, 630 fungal strains were isolated from symptomatic wheat samples collected from 76 fields in the wheat-growing regions of Azerbaijan during the 2017 growing season. As a result of the classification of species based on morphological and molecular tools, 443 isolates of *Fusarium* spp. were identified (Table 1). Meanwhile, 1221 fungal isolates were isolated during the 2019 growing season from symptomatic wheat samples collected from 65 fields in Kazakhstan's wheat-growing regions. Based on morphological and molecular tools, 537 of *Fusarium* spp. were identified (Table 2).

Table 1. The number of isolates associated with wheat crown/root rot and isolation frequency obtained from eight different wheat-growing regions in Azerbaijan

Azarbaıjan	**Number of isolates associated with crown/root rot of wheat from the locations**									**Rate of total isolates %**
	Qobustan	**Ismailli**	**Oguz**	**Shaki**	**Barda**	**Agdash**	**Ujar**	**Kurdamir**	**Total**	
Fusarium culmorum	0	0	5	18	43	8	9	109	192	43.3
F. acuminatum	3	19	9	46	7	3	4	34	125	28.2
F. graminearum	0	0	0	5	3	0	13	22	43	9.7
F. equiseti	0	0	0	15	0	0	0	17	32	7.2
F. hostae	0	6	5	0	0	0	0	0	11	2.4
F. profileratum	0	0	0	0	0	0	0	9	9	2.0
F. avenaceum	0	0	5	0	0	0	0	2	7	1.5
F. oxysporum	0	0	0	5	2	0	0	0	7	1.5
F. pseudograminearum	0	6	0	0	0	0	0	0	6	1.3
F. incarnatum	0	0	0	0	4	0	0	1	5	1.1
F. algeriense	0	2	2	0	0	0	0	0	4	0.9
F. brachygibbosum	0	0	0	0	0	0	0	2	2	0.4
Total	**3**	**33**	**26**	**89**	**59**	**11**	**26**	**196**	**443**	**100**

Table 2. The number of isolates associated with wheat crown/root rot and isolation frequency obtained from three different wheat-growing regions in Kazakhstan

Kazakhstan	Number of isolates associated with crown/ root rot of wheat from the locations				Rate of total isolates %
	Karagandy	East Kazakhstan	Almaty	Total	
Fusarium acuminatum	158	39	52	249	46.3
F. equiseti	26	48	50	124	23.0
F. culmorum	14	22	22	58	10.8
F. oxysporum	5	21	24	50	9.3
F. redolens	4	19	6	29	5.4
F. pseudograminearum	2	2	5	9	1.6
F. flocciferum	0	0	9	9	1.6
F. incarnatum	4	0	0	4	0.7
Fusarium sp.	1	0	2	3	0.5
F. torulosum	1	1	0	2	0.3
Total	**215**	**152**	**170**	**537**	**100**

For Azerbaijan samples, *Fusarium* spp. strains were found at a frequency of about 70.3%. The *EF1*-α gene sequences of 64 representative *Fusarium* spp. isolates ranged from 589 to 727 bp long, with identity matches of 99 to 100% with those of corresponding *Fusarium* spp. in the GenBank database. *Fusarium culmorum* and *F. acuminatum* were predominant *Fusarium* spp., with frequencies of 43.3 and 28.2%, respectively, among *Fusarium* spp. isolates, followed by *F. pseudograminearum* and *F. equiseti*, with frequencies of 9.7 and 7.2%, respectively. *F. hostae*, *F. proliferatum*, *F. avenaceum*, *F. oxysporum*, *F. graminearum*, *F. incarnatum*, and *F. algeriense* were found less commonly, whereas *F. brachygibbosum* was the least predominant.

The pathogenicity of *Fusarium* species were tested for their ability to cause disease. *Fusarium culmorum, F. pseudograminearum*, and *F. graminearum* isolates caused severe disease on crown and root of wheat plants with means of 3.52, 3.49, and 3.23 disease severity scores, respectively, and were considered to be highly virulent species. Some isolates of these species caused severe damage and resulted in the death of whole plants in some pots. *Fusarium avenaceum, F. algeriense,* and *F. hostae* produced moderate disease severities. *Fusarium acuminatum, F. incarnatum, F. equiseti, F. oxysporum, F. brachygibbosum,* and *F. proliferatum* isolates exhibited no significant differences from control plants and were determined to be nonpathogenic.

In the Kazakhstan isolates, *Fusarium* isolates comprised 43.98% of the total number of isolates and were classified into 10 *Fusarium* species by comparing them to the published descriptions and DNA sequencing. The EF1-α gene sequences of *Fusarium* spp. isolates ranged in length from 636 to 693 bp, with 100% identity matches to corresponding *Fusarium* species (except *Fusarium* sp.) in the GenBank database. *Fusarium acuminatum* and *F. equiseti* were the most frequent *Fusarium* species, accounting for 46.37% and 23.09% of *Fusarium* spp. isolates, respectively, followed by *F. culmorum* and *F. oxysporum*, which accounted for 10.80% and 9.31%, respectively. *Fusarium redolens*, *F. pseudograminearum*, *F. flocciferum* (Corda), *F.* cf. *incarnatum*, and *Fusarium* sp. were found less commonly, with frequencies of 5.40%, 1.68%, 1.68%, 0.74%, and 0.56%, respectively, whereas *F. torulosum* was the least predominant (0.37%), and two isolates could be identified in one field from Karagandy and one field from East Kazakhstan. Except for *F. flocciferum* (4.62% incidence), *Fusarium* sp. (3.08% incidence), and *F.* cf. *incarnatum* (3.08% incidence), other *Fusarium* species could be found in each of the three surveyed regions, but the incidence of individual species varied among these regions. Concerning individual species, *F. acuminatum* was found in 66.15% of the surveyed fields, *F. oxysporum* in 41.54%, *F. equiseti* in 35.38%, *F. culmorum* in 18.46%, *F. redolens* in 9.23%, and *F. pseudograminearum* in 7.69%.

The pathogenicity tests revealed that *F. pseudograminearum* and *F. culmorum* isolates proved to be the most and equally virulent pathogens ($p < 0.05$) and had a mean crown rot severity of 3.55 and 3.42, respectively. Several isolates of both species caused substantial damage, with entire plants dying in some pots. *Fusarium* sp. isolates were moderately virulent on the crowns and roots, inducing necrosis or rot at similar levels. Likewise, *F. redolens* isolates showed a mean crown rot severity of more than 2 and were moderately virulent but not as severe as the species. *Fusarium acuminatum*, *F. oxysporum*, *F. flocciferum*, *F. torulosum*, *F.* cf. *incarnatum*, and *F. equiseti* were non-pathogenic on wheat seedlings, displaying no significant differences from control plants.

Accurate identification of isolates and analyses of pathogenic variabilities within the pathogen populations provide critical information for control strategies and breeding schemes. Species identification of the genus *Fusarium* based on morphological characteristics are time consuming and laborious and pose difficulties such as high morphological similarities and difficulty to induce sporulation. To overcome these bottlenecks, molecular phylogenetic analyses based on ITS and EF1-α sequences have been widely used for the identification of different species in this genus. The EF1-α locus was used to identify *Fusarium* spp. obtained from diseased wheat plants in Türkiye and China (Shikur Gebremariam et al. 2018; Xu et al. 2018). The sequences of ITS and the largest subunit of RNA polymerase (RPB1) sequences were necessary for the precise identification of *F. algeriense* isolates.

In all, 11 of 17 fungal species determined in this study were identified as *Fusarium* spp. *F. culmorum* was found to be the most predominant species occurring on crown and root tissues of wheat with 192 isolates and was detected in 57.9% of the fields surveyed. Among the complex of *Fusarium* spp. associated with FCR, *F. culmorum* has also been reported as the most frequently isolated species in the United States (Moya-Elizondo et al. 2011; Strausbaugh et al. 2004), New Zealand (Bentley et al. 2006), eastern Australia (Burgess et al. 1981), Türkiye (Tunali et al. 2008), Norway (Kosiak et al. 2003), Latvia (Bankina et al. 2013), Chile (Moya-Elizondo et al. 2015), and Algeria (Abdallah-Nekache et al. 2019). This present study was also consistent with previous observations that *F. culmorum* prefers cooler areas. We observed that *F. acuminatum* was the second most commonly isolated *Fusarium* sp. from below ground, with 125 isolates representing about 55.3% of the fields surveyed. The ratio of *F. acuminatum* with 125 isolates to total isolates was 19.8%. This agreed with the result of a survey conducted in Türkiye that *F. acuminatum* had close isolation frequency with *F. culmorum* at 14% (Shikur Gebremariam et al. 2018). *F. acuminatum* fungus has been found in association with *B. sorokiniana* in the root-disease complex, especially in dryland wheat production areas of Colorado and Wyoming (Fernandez et al. 1985; Gonzalez and Trevathan 2000; Hill et al. 1983; Specht and Rush 1988). Similar to *F. culmorum*, there are many reports that this fungus prefers cold weather, which is a common climatic condition in the survey area (Hall and Sutton 1998; Pettitt et al. 2003). *F. pseudograminearum* and *F. equiseti* were represented with 43 and 32 isolates, respectively. *F. pseudograminearum* (formerly known as *F. graminearum* group I) was isolated from 14.5% of the fields surveyed in the wheat-growing area of the country. The prevalence of this fungus has been reported to increase especially in arid and low-rainfall areas and years (Poole et al. 2013; Smiley and Patterson 1996), which explains why fungi were mostly obtained from Ujar and Kurdamir. *F. equiseti* has been reported as a dominant fungus associated with crown and root rot of wheat in Saskatchewan (Fernandez and Jefferson 2004), Mississippi (Gonzalez and Trevathan 2000), Italy (Rossi et al. 1995), Canada (Fernandez et al. 2014), and Türkiye (Shikur Gebremariam et al. 2018), which confirms our results.

Global food production continues to be challenged by an alarmingly growing population, a finite amount of arable land, and conflicts between the countries that are important in the world wheat trade. Wheat is severely threatened by rapid climate change, which affects the world's abiotic and biotic components. In an effort to address some of these challenges, field assessments of the crown and root rot pathogens on wheat, which harbors an array of pathogenic species, are reported on a regular basis in the world's largest wheat-producing countries. This is the first detailed survey on pathogens of underground parts of wheat in Azerbaijan and Kazakhstan. The identification of species was conducted with morphological and molecular techniques. Pathogenic characterization was employed with Seri 82 to reveal the aggressiveness of the pathogens to wheat. The findings of this research will be useful for the development of management guidelines for root and crown rot fungi in wheat across Azerbaijan and Kazakhstan.

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Preliminary screening for resistance to Fusarium foot and root rot on old Tunisian durum wheat (*Triticum durum* Desf.) accessions

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Summary

Fusarium foot and root rot (FFRR) caused by *Fusarium culmorum* is an important soil borne disease of cereals in the dry land regions including Tunisia and durum wheat appears to be the most susceptible species. As no sources of resistance has been detected on this species, this disease has been the subject of intensive screening and genetic studies. In this context, 100 old durum Tunisian wheat accessions issued from the Tunisian national breeding program were screened at seedling and adult stages during 2021-2022 growing season. Two trials were setup; in pots for the seedling stage trial and in field for adult stage, using a bread wheat variety as a resistant control and five commercial durum wheat varieties as susceptible negative controls. All plants were inoculated by *F. culmorum*. Disease severity (DS) was assessed according to a scale ranging from 0-7 on both seedlings and adult plants. while, whiteheads (WH) rate, the heading date (HD) and plant height (PH) were estimated on adult plants. In terms of DS, the positive control expressed a higher resistance to the disease *vs* the five negative controls on both seedling and adult plant. Basing on the lowest DS expression *vs* the positive control, on the 100 old durum wheat accessions, five of the them at seedling stage and 14 at adult stage showed better disease resistance to FFRR. Parallelly, 10 varieties at seeding stage and 18 at mature stage, expressed a higher resistance *vs* the five negative tested control. In addition, at maturity stage, among the 14 varieties, 7 showed increased resistance *vs* positive control based on both DS and the WH parameters. In addition, the bread wheat positive control has an early HD and a shorter PH *vs* all negative controls. However, these two parameters could not explain the behavior of the superior resistance obtained on the old durum wheat accessions *vs* bread wheat in this work. Thus, more replication and investigation are needed.

Introduction

Durum wheat is considered as the most important food crop in Tunisia as up to 50% of the total cultivated cereal areas is devoted to this crop (Tunisian Ministry of Agriculture 2022). It is mainly grown under irrigated environments and rainfed climate of north west regions. Currently with the frequent climatic changes in these regions, soil borne disease, including Fusarium foot and root rot (FFRR) is becoming the most important and devastating challenging durum wheat production through its effects on grain yield (Daniel and Simpfendorfer 2008; Chekali et al. 2013).

The disease is mainly caused by *Fusarium pseudogramiearum* (O'Donnell and T. Aoki; group I) (= *Gibberella coronicola*) and *F. culmorum* (W.G. Smith) Sacc. (Burgess etal. 2001; Miedaner etal. 2008; Hogg etal. 2010; Moya-Elizondo et al. 2011). *F. culmorum* which is characterized by a higher virulence *vs F. pseudograminearum* (Papendick and cook 1974; Cook, 1981; Ozdemir et al. 2020) is the most common species in Tunisia (Gargouri et al. 2001; Boughalleb et al. 2008; BenSassi et al. 2011). This constitutes an additional challenge for the disease management. Furthermore, no source of resistance has been identified in durum wheat. In fact, several works have shown that there is no absolute resistance against Fusarium, and the term 'resistance' used in this context refers only to 'partial resistance'. this partial resistance, was identified in several bread wheat germplasm (Erginbasorakci et al. 2018; Yang et al. 2019; Jin et al. 2020 ; Pariyar et al. 2020 ; Jun e al. 2021;Yang et al. 2021) but not on durum wheat (Wallwork et al. 2004; Hollaway et al. 2013; Kemal et al. 2018; Jin et al. 2021).

Investigation for the resistance to FFRR is complex and seems to be closely linked to environment as well as agro morphological plant characters. In Tunisia, except for Gargouri et al. (2009) and Chekali et al. (2013) there is not much information on the behavior of durum bread wheat germplasm to FFRR. Recently, a study on the behavior of commercial durum and bread wheat varieties was carried out over two years and showed that bread wheat is more resistant than durum wheat (results not published). In this context, the current study was set up to highlight the performance of old durum wheat accessions, bread wheat and commercial durum wheat varieties to FFRR.

Methods

Experimental site description. The trial was conducted at the Experimental Station of the Tunisian National Agronomic Institute (INRAT) in Kef. The station is located in northwest of Tunisia (36°7'N; 8°43'E), at 780 m elevation. The soil is clay (50%), loam (30%) and sandy (20%). The climate is Mediterranean, with cold humid winter and very hot dry summer.

Plant and inoculum. A number of 100 old Tunisian durum wheat accessions issued from the Tunisian national breeding program were compared to one commercial bread wheat (Haidra) resistance positive check and five commercial durum wheat (Selim, Maali, Karim, Dhahbi et INRAT100) susceptible negative check. Inoculum was produced on sterilized oat colonized with three isolates *F. culmorum* (FC3, FC4, FC8) of Pôle Regional de Recherche Developpement du Nord Ouest Semi Aride (PRRDANOSA). Grains of oat were soaked in water overnight and then excess water was drained off. One liter Erlenmeyer flasks were filled with 250 ml of oat grains, sealed with cotton wool and covered loosely with aluminum foil. The flasks were autoclaved at 120°C for 20 min twice over 2 days. After cooling, the oat grains were inoculated aseptically with 8 segments (1 cm × 1 cm) from a 9 cm plate of each isolate of *F. culmorum* grown on PDA and incubated for 14 days at 25°C. The colonized grains were air-dried on filter paper, ground in a laboratory mill and passed through a 2 mm sieve.

Experimental trial. The trial was set up as an Augmented block Design with five blocks during 2020-2021 growing season. For the seedling stage test, 130 pots with a capacity of 10 g each, were filled with soil inoculated at 1% (0.1 g of inoculum per 10 g of soil). The pots were filled to ¾ of their capacity, then two seeds of each variety were placed and then covered with the rest of the soil. They were placed outdoor and watered regularly. For adult stage test, conducted in open field, 130 twin rows of 1 m spaced 0.17 m apart were sown by 10 seeds/m ×2, for each variety and at the Zadoks 21 stage, 1.25 g/m of inoculum was sprinkled directly at the base of the plants.

***Disease assessment*.** Plants were moved from soil and the disease severity was estimated at Zadoks stage 13, for the seedling test and Zadoks stage 55, for plant mature test according to scale ranging from 0-7. For seedlings, Index 0: no symptoms on the roots with a clear stem ; index 1: light browning on the stem base ; Index 2: light browning on the stem base and beginning of brownish of the first leaf; Index 3: light browning on stem base, the first leaf and beginning on the second leaf ; Index 4: light to dark browning on the stem and first leaf, and extends on the second leaf; Index 5: intense browning of the stem and total necrosis of the first leaf and the second leaf; Index 6: total necrosis of the seedling with total destruction of the roots; index 7: no plant emergence. For mature plants, Index 0: no symptoms on the roots with a clear stem; index 1: slight browning on the stem base; Index 2: slight browning on the first internode; Index 3: Extension of the browning on the second internode with a more intense browning on the first internode; Index 4: extension of browning to the third internode; Index 5: extension of the browning towards the fourth internode; Index 6: intense browning from the first to fourth internodes with a puny appearance of the plant; Index 7: total browning of the plant. Besides, whiteheads parameter was evaluated by heads sensing prematurely.

Agro-morphological plant assessment. the heading date was estimated after 50% emergence of the plant's heads and the plants height average were measured at plant maturity plants stage (Zadoks 89).

Statistical analyses. Data were analyzed using ANOVA to test the significance of main effects using R software. The old durum wheat accessions and the controls were considered as main factors. Means comparisons was performed using Duncan's LSD tests ($P = 0.05$ or 0.01).

Results

In terms of DS, data analyses, showed that difference between the positive and negative controls was almost significant ($P < 0.07$) at seedling stage and significant ($P < 0.01$) at mature stage. Besides, difference between positive control and the old durum wheat accessions tested was a highly significant ($P < 0.0001$) at seedling stage and almost significant ($P < 0.07$) at mature stage. At seedling stage, the five old durum wheat accessions (71, 83, 42, 40, 89), behaved globally better *vs* the positive control with accessions 71 expressing the lowest DS. In addition to these accessions, five others (86, 91, 97, 54, 5) expressed lower DS *vs* all negative controls (Figure 1).

At maturity stage, on 14 old durum wheat, two accessions 4 and 6 had significantly lower DS *vs* the positive control while 64,81,84,53,56,17,18,35,77,78,80,99 expressed a similar behavior. These 14 varieties and 17 others (49, 51, 60, 1, 12, 16, 20, 9, 33, 65, 68, 71, 79, 93, 95, 96, 97, 98) had better behavior than all durum wheat negative controls (Figure 2).

Regarding white heads, parameter evaluated at adult stage, 32 of old durum accession (39, 38, 36, 35, 33, 32, 27, 25, 24, 9, 7, 6, 4, 3, 20, 2, 18, 17, 16, 12, 11, 1, 98, 97, 96, 95, 93, 92, 91, 84, 82, 81) didn't show this symptom *vs* positive control. In addition, seven accessions (4,6,35, 18,17,84,81) showed increased resistance *vs* positive control based on both DS and the WH parameters.

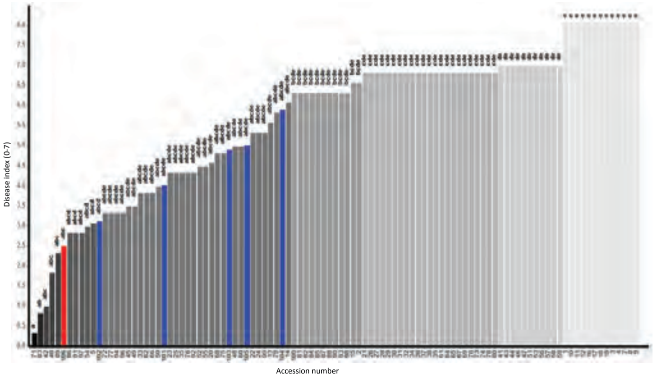

Figure 1. Disease severity on old Tunisian durum wheat accessions *vs* bread wheat variety as positive control (Red bar) and commercial durum wheat varieties as negative controls (blue bars) at seeding stage.

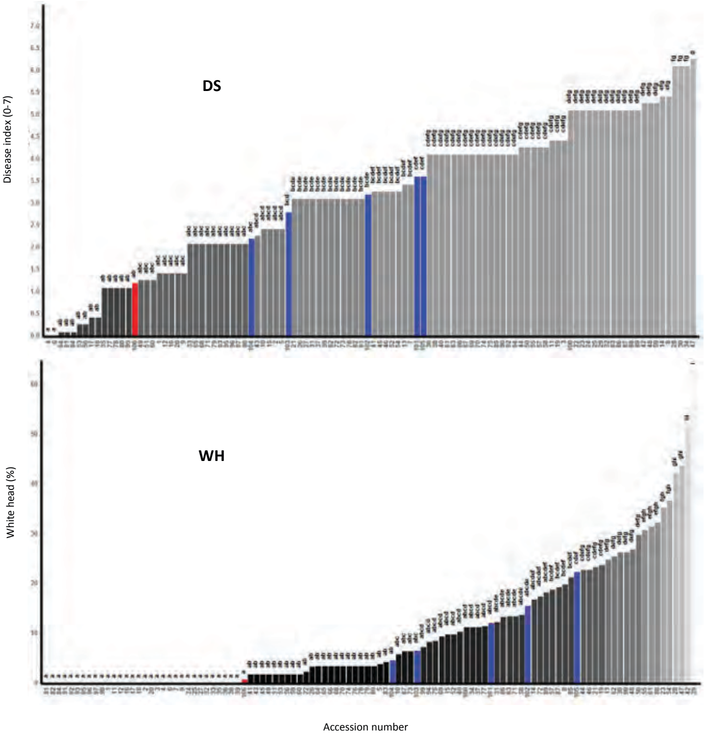

Figure 2. Disease severity (DS) and whiteheads (WH), evaluated on old Tunisian durum wheat accessions *vs* a bread wheat variety as positive control (red bar) and commercial durum wheat varieties as negative controls (blue bars) at maturity stage.

Based on HD and PH plant, the resistant positive control headed early and was shorter compared to susceptible negative control. However there was no interaction between these two parameters and the superior reaction found on the 14 old varieties of durum wheat accessions screened at mature stage.

Discussion

Investigation of wheat resistance to FFRR is a challenging process. In this framework, some Tunisian bread wheat lines are resistant in pots (Gargouri et al. 2009) and are more tolerant to the disease *vs* durum wheat in dry conditions (Chekali et al. 2013). However commercial durum wheat varieties are globally susceptible (Chekali et al. work not published). To find out additional information, the present work has focused on the behavior of 100 old Tunisian durum wheat accessions *vs* bread wheat and some commercial durum wheat controls at seedling and mature stages. The behavior between controls used in the present work, confirms that bread wheat has a superior resistance to the Tunisian commercial durum wheat varieties at seedling and mature stage which is in accord with several finding such as those of Wallwork et al. (2004), Kazan and Gardiner (2018) and Yang et al. (2021). This work has also shown that screening for resistance against FFRR does not give the same results at the seedling *vs* maturity stage of plant. Indeed, on the 100 old durum wheat accessions tested, the few accessions which showed greater resistance *vs* positive control at the seedling stage were not the same as at the maturity stage. This may suggest that the resistance found in durum wheat is probably multigenic, dependent on other factors. In light of this, relation between resistance *vs* height and heading date of plants were investigated. It was found that bread wheat positive control was shorter and more resistant to the disease and heads earlier *vs* the five susceptible durum wheat controls. This is in agreement with Liu and Ogbonnaya (2015); Ma et al. (2010); Yang et al. (2010); Zheng et al. (2014) and Krishnamurthy et al. (2013) findings. surprisingly, this has not been confirmed on durum wheat old accessions expressing a higher resistance *vs* bread wheat. The resistance of some old accessions of Tunisian durum wheat obtained in this work seem to be interesting and deserves to be confirmed over more years by taking into account the agro- morphological or environmental factors linked to this resistance. In addition, the unavailability of correlation between screening resistance to FFRR at the seedling and adult stage, must be taken into account in national breeding programs.

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Preliminary studies on biological control of wheat root rot diseases

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Summary

This study was carried out to determine the *in vitro* antagonistic potentials of some endophyte and rhizosphere bacterial microbiomes against wheat root rot disease casual agents such as *Fusarium culmorum* W.G. Smith, *Drechslera sorokiniana* (Saccardo) Subramanian & Jain, *Gaeumannomyces graminis* [Sacc.] Arx & Oliver var. *tritici* [J. Walker] (*GGT*), which are highly destructive on wheat production areas and causing important economical loses. Inhibition of mycelial growth of major foliar and soil borne fungal disease agents were investigated. A total of 141 bacterial biocontrol agent were studied for their biocontrol activities. Among them, 31 bacterial isolates (21.98% of total isolates) inhibited mycelial growth of *F. culmorum* by 96.1%, *GGT* by 100% and *D. sorokiniana* by 89.9%, respectively. A majority of bacterial isolates were identified as *Bacillus* spp. Certain isolates of *Bacillus* spp. caused noticeable morphological changes on mycelia of *F. culmorum*, *GGT* and *D. sorokiniana*. Antagonistic potentials of bacterial isolates were found to increase by pre-incubation time prior to fungal inoculation. Due to high antagonistic properties, efficient isolates of *Bacillus* spp. may be used as biocontrol agents against soil borne diseases as an alternative to pesticides to promote sustainable agriculture.

Introduction

Wheat (*Triticum* spp.) is the most produced product in the grain fields of Türkiye. According to 2019 data, wheat is grown on 7.7 million hectares, barley on 2.7 million hectares and corn on 0.7 million hectares of total grain areas in Türkiye. Wheat, which ranks first with a production of 20.6 million tons in cereals with a total production of 35 million tons, constitutes 55.2% of the grain production. The wheat yield in 2019 was 25-30 kg/ha (Anonymous 2020).

Abiotic and biotic stress factors are the most important factors that negatively affect the yield and quality of wheat. Pathogens, one of the biotic stress factors, cause diseases in plants, affect yield and quality significantly and cause economic losses. Among them, soil-borne pathogens constitute the most important group.

Root and crown disease agents are generally soil-borne. They are also carried by seeds and plant residues. It is very difficult to combat these factors due to their ability to survive in the soil for many years. It is possible to encounter root and crown rot diseases in all development stages of the plant. While most of the disease factors can originate from seeds and soil, their importance increases even more because they cause different diseases in leaves and spikes other than the root and crown of the plant. For example, *Fusarium* spp. cause head blight and *Dreschlera* spp., *Fusarium* spp., *Alternaria* spp. can also attack above ground parts. They can also cause embryo darkening (black spot) disease.

Root and crown rot diseases are the leading pathogen groups affecting wheat and barley yield in terms of quality and quantity. In order for the plants to develop normally and give the best yield, they must have a healthy root system. Disease agents can cause damage to most of the grass crops. These include wheat, barley, triticale, oats and rye. Generally, root and crown rot disease factors in wheat and barley plants reduce the number of plants per square meter, the number of heads per plant, the length of the spikes, and therefore the number of grains per spike. These factors, first of all, are effective in the root and crown parts of the plant, thus reducing the nutrient and water absorption capacity of the plant roots. Root and crown rot disease agents such as *Fusarium culmorum* and *Gaeumannomyces graminis* (*GGT*) cause some spikes to take on a white appearance during the growing period in the fields where the disease is intense. Such spikes cannot form grains or form weak grains. Thus, the disease factors cause damage in terms of quality by reducing the hectoliter weight of the grain. It has been determined by some researchers that the grain weight in the ear decreased by 30-60% due to root and crown diseases, which were quite common in Thrace in 1978, and the 1000-grain weight of the product obtained from the diseased field decreased by 17% compared to the healthy ones. *Fusarium* and *Rhizoctonia* species are the most common and important soil-borne fungi that cause root and root collar diseases in Turkish wheat fields, and the crown disease caused by *GGT* has also been a problem in recent years.

GGT, on the other hand, causes the disease known as take-all in wheat, barley and triticale. Although its main host is wheat, it causes disease in barley, triticale, rye, other grains and grasses and is considered as one of the most important root diseases affecting wheat yield in many parts of the world (Cook 1992). The fungus can infect plant roots and rot and destroy the entire root system. The root and rhizome turn charcoal black and are easily seen when the plant is pulled. The fungus invades the stem upwards, causing the plant to collapse. The pathogen lives in the infected root and stem of the plant. Infection persists throughout the growing season. The optimum soil temperature for the disease is 10-20 °C. The disease-causing fungus grows well in neutral to alkaline soils, sloping lands, poorly drained fields, and soils poor in nitrogen and phosphorus (Wiese 1998). It was later reported that it was detected in Sakarya (Aktaş et al. 1996). With the climate change in recent years and the planting of susceptible varieties, the disease has spread to the Central Anatolia Region (Aktaş et al. 1999, Büyük et al. 2018, Tunalı et al. 2008) and Thrace (Hekimhan 2010) and has created serious problems.

Chemical control of *GGT* in the world is limited and difficult. Currently, there is no licensed plant protection product against the disease in Türkiye. It has been reported that the disease causes more than 40% product loss in wheat if it is not controlled (Genowati 2001). Due to the limited chemical control and the harmful effects of chemicals, alternative methods of combating this disease have gained importance. One of the most important of these methods is the use of disease-resistant varieties and alternative methods like biological control.

Erdurmuş and Katırcıoğlu (2008) investigated the inhibition and effects of *Trichoderma harzianum* isolates against wheat root and crown pathogens (*Fusarium culmorum*, *F. pseudograminearum*, *Bipolaris sorokiniana* and *Rhizoctonia solani*) in sterile and natural soil conditions in 2006. In the inhibition trial, T10 isolate showed 82.6% inhibition against, *F. culmorum,* against *F. pseudograminearum* T7 72.2%, against *B. sorokiniana* T7 76.4%, and against *R. solani* T1 67.8% inhibition. *T. harzianum* isolates were applied to the wheat seed of Kınacı 97 variety as a coating. In sterile soil, T10 was 65.6% effective against *F. culmorum*, T4 51.9% against *F. pseudograminearum*, T6 63.5% against *B. sorokiniana*, T5 73% against *R. solani*, and T3 23.3% against *F.*

culmorum in natural soil. T6 inhibited *F. pseudograminearum* by 15.8%, *B. sorokiniana* by 60.4% and T10 inhibited *R. solani* by 26.8%.

Karahan et al. (2011) obtained 105 non-pathogenic fluorescent *Pseudomonas* isolates from Ankara, Eskişehir and Sakarya provinces in 2007-2009. In *in vitro* tests, they measured the inhibition zones created by antagonistic bacteria against the pathogen *F. culmorum* in petri dishes containing Potato Dextrose Agar (PDA). 67 of them were identified as weak antagonists (zone of inhibition <5 mm). Three of the antagonist bacterial isolates were *Pseudomonas fluorescens*, one was *P. putida*, and two were *Pseudomonas* spp. In *in vivo* trials, it has been reported that none of the bacterial isolates showed antagonistic activity against the pathogen *F. culmorum*.

Gökçe (2013) did a master's study named "Investigation of biological control possibilities under controlled conditions by using PGPR and bio agent bacteria against *Bipolaris sorokiniana* (Sacc.)", which causes wheat root rot. Using PGPR and bioagent bacteria, the control of wheat root rot disease *B. sorokiniana* and its effect on plant growth were determined. In the study, a total of 212 antagonist bacterial isolates belonging to 25 different species identified by Microbial Diagnosis System (MIS) were used, isolated from underground or above-ground parts of wild and cultivated plants in different previous studies. These antagonist bacteria were tested in vitro to determine their antifungal properties against the pathogen. 39 antagonist bacterial isolates, selected considering their antifungal properties, nitrogen fixation and phosphate dissolution properties, were developed in liquid carrier formulation. They were tested in terms of their effectiveness on disease development and growth parameters of wheat plants in pot trials using seed coating method. It was determined that many of the antagonistic bacteria were both effective in suppressing the disease and making important contributions to plant growth. As a result, it has been determined that some of the liquid formulations (*Bacillus megaterium* TV-6D, *Brevibacillus choshinensis* TV-53D and *Bacillus pumilus* TV-3C) formed from antagonist bacterial isolates can be used both as bio pesticides in the control of wheat root rot disease and as microbial fertilizers in wheat cultivation.

Methods

Pathogens (*F. culmorum*, *GGT* and *D. sorokiniana*) used in this study were provided by the Cultural Collection of Plant Pathogens from Plant Protection Central Research Institute/Ankara. Antagonistic bacterial isolates were provided from the previous studies conducted by Dr. Kamil DUMAN from soil, seed and plant tissue samples.

Determination of antagonistic effects of isolated antagonist bacteria (antb) isolates on mycelial growth of fungal agents

The potential of selected candidate BCA bacterial isolates to inhibit (antagonize) mycelial growth of disease agents was determined by double culture tests in petri dishes containing PDA, as previously reported (Soylu et al., 2005). In these tests, the bacterial isolate to be tested was drawn on the tip of each petri dish, and after pre-incubation at 26°C for 24 hours, 5 mm diameter mycelial discs taken from the growth areas of fresh pathogenic fungus cultures developed in PDA medium. They were placed in the middle so that they were equidistant from the candidate antagonist bacterial isolates and incubated at 26°C. No antagonist bacterial isolate was placed into the control dishes. Upon reaching the 75% growth in the control plates, the fungal mycelium growth (MGT) directed towards the bacteria was measured (3-7 days after inoculation depending on the development of the fungal agents) and the % inhibition rates according to the mycelial growth (MGC) in the control plates were calculated using the formula given below.

% Inhibition = ((MGC-MGT)/MGC) *100. For each bacteria-fungi combination, measurements were made in three different petri dishes, and the experiment was repeated at two different times.

Experimental design and statistical analysis

All trials were set up according to the randomized blocks trial design, without converting the inhibition rates of pathogen development to %, using the SPSS statistical program (SPSS Statistics 17.0). Analysis of Variance was performed with one-way ANOVA and the difference between isolates was determined by Duncan's Multiple Range Test. ($p \leq 0.05$).

Results and discussion

In *in vitro* efficacy studies, it is thought that these isolates of *Bacillus* spp. can be directly converted into a tool (biological preparation) that can be used to prevent the losses caused by disease agents in different crops.

Among 141 different candidate antagonist bacterial isolates, 27 of them showed significant inhibition performance which is over than 50% of them, and they caused mycelial destruction. 114 of them showed less inhibition but the most important factor that caused the separation of the 27 significantly successful bacterial isolate was they sustained the rate of inhibition even after 21 days after dual culture assay. The rest of the candidate bacterial isolates did not show inhibition zones; after 7-14 days the inhibition zone was covered by the pathogen fungi.

When the effectiveness of the tested candidate antagonists on the inhibition of mycelial growth of *F. culmorum* was examined, isolate AntB5 was the most effective isolate with 96.1% inhibition rate, this isolate is followed by isolate AntB18 with a 94.9% inhibition rate. The licensed plant protection product with *B. subtilis* as an active ingredient (AntBKU) was used as a comparative application, and caused 94% inhibition. The candidate antagonist isolate that inhibited *F. culmorum* mycelial growth at the lowest rate was isolate AntB15 with a rate of 56.9% (Table 1).

When the effectiveness of the tested candidate antagonists on the inhibition of mycelial growth of *GGT* was examined, AntB5 isolate was the most effective isolate with 100% inhibition rate, this isolate was followed by AntB7 isolate with a 99.7% inhibition rate, and the licensed plant protection product (AntBKU) with active *B. subtilis* showed 100% inhibition. The candidate antagonist isolate that inhibited *F. culmorum* mycelial growth at the lowest rate was AntB15 isolate with a rate of 49.7% (Table 2).

When the effectiveness of the tested candidate antagonists on the inhibition of mycelial growth of *D. sorokiniana* was examined, AntB5 isolate was the most effective isolate with 89.9% inhibition rate, this isolate was followed by AntB8 isolate with a 89.8% inhibition rate, and the licensed plant protection product with active with *B. subtilis* (AntBKU) as a comparative application caused 84.4% inhibition. The candidate antagonist isolate that inhibited *F. culmorum* mycelial growth at the lowest rate was AntB15 isolate with a rate of 53.9% (Table 3).

Table 1. Inhibition percentage of the antagonist bacterial isolates against *F. culmorum* isolate on petri dishes by dual culture assay

Isolate	***F. culmorum*** **Inhibition (%)**
AntB5	96.1±0.4 ^{A}
AntB18	94.9±0.7 ^{AB}
AntBKU	94.0±0.1 ^{AB}
AntB14	93.1±0.4 ^{AB}
AntB12	63.1±0.6 ^{EF}
AntB13	62.6±5.2 ^{EF}
AntB9	62.4±8.1 ^{EF}
AntB15	56.9±2.7 ^{F}
Control	**0±0.0** ^{G}

*Mean values followed by different letters within each column were significantly different according to Least Significant Difference (LSD) test (P≤0.05)

Table 2. Inhibition percentage of the antagonist bacterial isolates against *GGT* isolate on petri dishes by dual culture assay

Isolate	***GGT*** **Inhibition (%)**
AntB5	100±0.0 ^{A}
AntBKU	100±0.0 ^{A}
AntB7	99.7±0.3 ^{A}
AntB18	99.5±0.3 ^{A}
AntB2	99.0±0.5 ^{A}
AntB13	56.4±6.1 ^{FG}
AntB9	56.1±9.4 ^{FG}
AntB15	49.7±31 ^{G}
Control	**0±0.0** ^{H}

* Mean values followed by different letters within each column were significantly different according to Least Significant Difference (LSD) test (P≤0.05)

Table 3. Inhibition percentage of the antagonist bacterial isolates against *D. sorokiniana* isolate on petri dishes by dual culture assay

Isolate	*D. sorokiniana* Inhibition (%)
AntB5	89.9±1.0 ^{A}
AntB8	89.8±0.2 ^{A}
AntB24	88.8±0.9 ^{A}
AntBKU	84.4±0.4 ^{AC}
AntB13	62.6±5.2 ^{GH}
AntB9	62.4±8.1 ^{GH}
AntB15	53.9±2.1 ^{H}
Control	**0±0.0 ^{I}**

* Mean values followed by different letters within each column were significantly different according to Least Significant Difference (LSD) test (P≤0.05)

Many *Bacillus* spp., besides producing antimicrobial compounds, have the ability to form spores that are resistant to adverse environmental conditions. These spores can be easily converted into biological preparations with appropriate technologies and used in the control of many plant diseases (Emmert and Hendelsmann 1999). By investigating the *in vivo* efficacy of these antagonists against the disease agents they are tested for, it will be possible to select the most suitable isolate for use as a biological preparation. In addition, studies to be carried out on the determination of the mechanisms used by antagonist bacterial biocontrol agents to prevent disease emergence will further increase the widespread impact of the studies.

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Table 4. Inhibition percentage of the antagonist bacterial isolates against all pathogen isolates by comparison on petri dishes by dual culture assay

Isolate	*F. culmorum* Inh%	*GGT* Inh.%	*D. sorokiniana* Inh.%
AntB1	81.2±1.0 ^{AD}	78.1±1.2 ^{CE}	81.2±1.0 ^{AF}
AntB2	90.3±0.5 ^{AB}	**99.0±0.5 ^{A}**	76.9±0.6 ^{AG}
AntB3	**92.5±0.2 ^{AB}**	**98.9±0.3 ^{A}**	81.9±0.7 ^{AF}
AntB4	70.2±2.4 ^{CF}	65.3±2.8 ^{DG}	70.2±2.4 ^{CH}
AntB5	**96.1±0.4 ^{A}**	**100±0.0 ^{A}**	**89.9±1.0 ^{A}**
AntB7	92.1±0.1 ^{AB}	**99.7±0.3 ^{A}**	80.4±0.6 ^{AF}
AntB8	89.8±0.2 ^{AB}	88.1±0.3 ^{AC}	89.8±0.2 ^{A}
AntB9	62.4±8.1 ^{EF}	56.1±9.4 ^{FG}	62.4±8.1 ^{GH}
AntB10	91.8±1.6 ^{AB}	**98.2±0.5 ^{AB}**	81.7±3.3 ^{AF}
AntB11	83.6±1.5 ^{AC}	80.8±1.7 ^{BD}	83.6±1.5 ^{AE}
AntB12	63.1±0.6 ^{EF}	56.9±0.7 ^{FG}	63.1±0.6 ^{GH}
AntB13	62.6±5.2 ^{EF}	56.4±6.1 ^{FG}	62.6±5.2 ^{GH}
AntB14	**93.1±0.4 ^{AB}**	**98.6±0.6 ^{A}**	83.7±0.6 ^{AD}
AntB15	56.9±2.7 ^{F}	49.7±3.1 ^{G}	53.9±2.1 ^{H}
AntB16	72.4±0.6 ^{CE}	67.8±0.7 ^{DF}	72.4±0.6 ^{BH}
AntB17	67.9±3.4 ^{DF}	62.5±3.9 ^{EG}	67.9±3.4 ^{EH}
AntB18	**94.9±0.7 ^{AB}**	99.5±0.3 ^{A}	87.8±1.7 ^{AB}
AntB19	67.1±4.9 ^{DF}	61.7±5.7 ^{EG}	67.1±4.9 ^{FH}
AntB20	92.6±0.4 ^{AB}	**98.9±0.3 ^{A}**	82.2±1.3 ^{AF}
AntB21	68.6±3.4 ^{CF}	63.3±3.9 ^{EG}	68.6±3.4 ^{DH}
AntB22	70.7±6.0 ^{CF}	65.8±7.0 ^{DG}	70.7±6.0 ^{CH}
AntB23	80.5±2.0 ^{BD}	77.2±2.4 ^{CE}	80.5±2.0 ^{AF}
AntB24	88.8±0.9 ^{AB}	86.9±1.0 ^{AC}	**88.8±0.9 ^{A}**
AntB25	91.1±1.0 ^{AB}	97.8±0.7 ^{AB}	79.7±1.9 ^{AF}
AntB26	90.1±0.8 ^{AB}	98.2±0.3 ^{AB}	77.6±1.7 ^{AG}
AntBKU	94.0±0.1 ^{AB}	100±0.0 ^{A}	84.4±0.4 ^{AC}
Control	0±0.0 ^{G}	0±0.0 ^{H}	0±0.0 ^{I}

* Mean values followed by different letters within each column were significantly different according to Least Significant Difference (LSD) test (P≤0.05)

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Reactions of Root Rot (*Fusarium nygamai* Burgess & Trimboli 1986) Disease in Some Oat Varieties

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Abstract

Oat is an important cereal crop used in human and animal nutrition in the world. Among the biotic factors that negatively affect the yield and quality of oats are soil-borne fungal pathogens such as *Fusarium* spp., *Rhizoctonia solani* Kühn, *Pythium* spp., and *Bipolaris sorokiniana* (Sacc.) Shoemaker. In this study, the reaction study of *Fusarium nygamai* Burgess & Trimboli 1986, one of the soil pathogens, against some oat cultivars was carried out with pot experiments, with a randomized experimental design with three replications. It was revealed that the Yazır variety, followed by the Yeniçeri variety were susceptible according to the results of trials with seed treated with *F. nygamai* and planting sterile seed into soil infested with *F. nygamai*. In addition, while the disease severity was found to be lower (46.67%) on Diriliş variety with seed treated with *F. nygamai*, it was observed that this oat variety was the most susceptible variety (53.33%) among the examined oat varieties when sterile seed was planted into infested soil. On the other hand, with the variety Faikbey, disease severity was found to be lower (26.67%) when seed was infested with the fungus, this was the most resistant among the examined oat varieties.

Introduction

Oat (*Avena sativa* L.) ranks 3th among the cool climate cereals in terms of cultivation and production in the world. It is the fourth placed in Türkiye. In recent years, oats have increased their importance in human nutrition in the world and started to be used in industry, which has led to an increase in the production areas of oats. In addition to abiotic factors, biotic factors adversely affect the yield and quality of oats. Root and crown disease agents are generally soil-borne, such as *Fusarium* spp. *Rhizoctonia* spp., *Dreschlera* spp., *Pythium* spp. pathogens. As a result of root and crown diseases in cereals, the plant grows poorly, and empty and white ears are formed. However, the spike is weakened, causing a decrease in hectoliter weight and 1000 grain weight. The weight of the grains in the spike decreased by 30-60% due to root and crown diseases that harmed wheat in Thrace in 1978 and the 1000-grain weight of the product taken from the diseased fields was 17% lower than the healthy ones (Finci, 1979).

In 1986, *Fusarium nygamai* Burgess & Trimboli was first diagnosed in Australia (Burgess and Trimboli, 1986) and later reported in Africa, China, Malaysia, Thailand, Puerto Rico and the United States. It has been reported that *F. nygamai* is found in plants such as sorghum, millet, beans, cotton and in soils with colonized plant residues. Also, *F. nygamai* has been reported to be a pathogen of *Striga hermonthica* (Del.)

Benth. *Fusarium* spp., which causes seedling blight and root rot of rice in Sardinia (Oristano, S. Lucia) region of Italy, (*F. moniliforme, F. proliferatum, F. oxysporum, F. solani, F. compactum* and *F. equiseti*) were isolated with *F. nygamai* together with other *Fusarium* species (Fisher et al. 1982). Balmas et al. (2007) inoculated isolates of *F. nygamai* and *F. moniliforme* from Arboria rice cultivar in the study they conducted in Sardinia. As a result of the study, it was observed that both *F. nygamai* and *F. moniliforme* reduced the emergence of rice seedlings, compared to the uninoculated control, with *F. nygamai* 33% to 59% and *F. moniliforme* 25% to 50%.

The most common species are *Bipolaris sorokiniana, Fusarium nygamai, F. equiseti, F. oxysporum, F. subglutinans, Periconia macrospinosa, Phoma macrostroma* and *P. medicaginis* from the fields where wheat and barley were grown in 3 different parts of Tanzania. It was observed that fungal species were obtained and *F. oxysporum* and *F. nygamai* were dominant in fungal isolates obtained from roots (Van DYK, 2003). In Poland, in research on winter wheat, triticale, rye, summer barley and oat crops, it was determined that the most common agents isolated from roots are *Fusarium* species (*F. culmorum, F. oxysporum, F. equiseti, F. avenaceum*), *Gaeumannomyces graminis, Aureobasidium pullulans, Microdochium nivale (Monographella nivalis), Rhizoctonia solani, Bipolaris sorokiniana* (=*Cochliobolus sativus*) and *Cylindrocarpon destructans (Nectria radicicola*) (Kurowski 2002). *Fusarium* species associated with Fusarium spike blight were observed in cereal fields in the Ontario region of Canada between 2001-2017. In this study, 9 species were identified from 8800 grains from 176 oat fields between 2008 and 2017. As a result of the study, it was reported that *F. poae* was dominant in 93% of the fields and 23.5% of the grains in oats and this factor represented 68% of the pathogen population (Xue et al. 2019).

Some studies on oat fungal diseases have been carried out in our country. Studies on *Fusarium* spp. in wheat, barley, rye and oats were carried out in Türkiye (Özer and Soran, 1991) and it was reported by Bremer (1948) that he found *Fusarium oxysporum, F. equiseti* and *F. culmorum* in our country. In the province of Antalya in 2015-2016, disease reactions were observed for stem rust (*Puccinia graminis f. sp. avenae*), crown rust (*Puccinia coronata f. sp. avenae*) and powdery mildew (*Blumeria graminis f.sp. avenae*) on 164 local oat genotypes collected from Antalya, Isparta, Burdur and Muğla provinces under natural infection conditions. In this study, Sarı, Kahraman, Fetih and Yeniçeri oat cultivars were more resistant to stem rust disease than other genotypes. With powdery mildew disease, Faikbey oat cultivar with 15 genotypes was more resistant than other genotypes. It was observed that genotypes 9, 33 and 37 and Sarı oat cultivar were more resistant than other genotypes against crown rust disease (Çalışkan et al. 2017). In 2016, 40 oat leaf samples collected from İzmir, in the Foça and Menemen locations, were examined and isolated from these samples. It was reported that *Alternaria longipes*, which causes leaf blight in oats, was determined as a result of both classical and molecular diagnosis (Hekimhan et al. 2020).

Materials and Methods

Materials

The main material of the study was *Fusarium nygamai*, which was obtained from the roots of the oat plants in the Edirne province, seven oat varieties (Diriliş, Faikbey, OTAG, Katmerli, Seydişehir, Yazır and Yeniçeri), Potato Dextrose Agar (PDA) medium used for the isolation and growth of the fungus,

Methods

Isolation study

In the study, infected parts of the oat root were cut in the laboratory from oat plant samples sent from Edirne province in 2021. They were surface disinfested (GIVE METHOD). Cultrues were then transfered to PDA and placed in the incubator at 25±2 °C. The identification of growing Fusarium species was made using classical and molecular methods.

DNA isolation and sequence analysis

Genomic DNA extraction was carried out in accordance with the Qiagen Plant Mini Kit (Madison, WI, USA) protocol from the mycelium of the fungi grown in PDA, which morphologically resembled *Fusarium nygamai*, obtained from the pure *Fusarium* species obtained as a result of the isolation study. The total DNA obtained was subjected to PCR programs using EF1-986R (5-TACTTGAAGGAACCCTTACC-3) and EF1-728F (5-CATCGAGAAGTTCGAGAAGG-3) primers (Elongation factor). In the PCR cycle program, 1 cycle for 5 minutes at 96 °C; 36 cycles were completed at 94 °C for 30 seconds, at 52 °C for 30 seconds, at 72 °C for 1.5 minutes, and finally at 72 °C for 7 minutes. The obtained PCR products were run on a 1.5% agarose gel. Then, the bands in the gel were visualized with the help of a transilluminator and sent for sequencing. Sequence analyzes of PCR products were performed by a commercial company. After the analysis, the raw sequence data were analyzed using Bioedit (Version 7.0) programs and the sequence data of these fungal isolates from NCBI were compared with the isolates of *Fusarium* species in the database by BLAST analysis.

Pathogenicity Test

For the pathogenicity test, *F.nygamai* was grown on PDA at 24°C under 15 hours light and 9 hours dark conditions. Surface disinfection of the seeds of seven cultivars was done separately for each cultivar. Then, these seeds were germinated in sterile germination cups in an incubator at 25±2 °C. Discs with a diameter of 5 mm were taken from the fungus. The fungus disc and the germinating oat seed were placed in a 90 mm diameter Petri dish containing PDA with a distance of 2 mm between them. *F. nygamai* and germinated seed on the PDA medium. A single fungal disc and germinated seed were placed in each petri dish. Each variety was studied separately in 3 replications and as a control. Only germinated seeds were used for the control study. Petri dishes were kept in a 25°C incubator for 7 days. At the end of the 7th day, seeds were evaluated for pathogenicity using the 0–5 scale (Ichielevich-Auster et al., 1985) (Table 1) (Figure 1).

Table 1. Evaluation scale for pathogenicity test (Ichielevich-Auster et al.,1985)

0	No signs of diseases
1	1-10% of the hypocotyl is infected
2	11-30% of the hypocotyl is infected
3	31-50% of the hypocotyl is infected
4	51-80% of the hypocotyl is infected
5	The all hypocotyl is infected

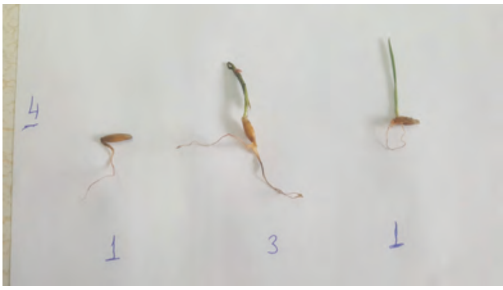

Figure 1. Result of pathogenicity test.

The results obtained from all pathogenicity tests were calculated according to the formula given below and % disease severity rates were found for each.

$$\% \text{ Disease Severity}: \frac{\Sigma(\text{Each scale value x number of samples in scale value})}{\text{Maximum scale value x Total number of plants}} \text{ x } 100$$

Planting Infected Seed with *F. nygamai*

Oat seeds in this study were disinfested separately 4-5 days before fungal inoculation. These seeds were germinated by placing them in a humidity chamber (nearly 60%) at 25-27 °C, with 12 hours of daytime/12 hours of night. In seed inoculation, petri dishes of *F. nygamai*, which were grown in PDA, were covered with sterile distilled water. Spores were transferred to the water with the help of a brush. Then a few drops of Tween 20 were added. The spore density was determined on a haeomcytometer slide to be 17 x 10^5 spores/ml. For inoculation of seven varieties of oat seeds with a fungal solution of this density, 30 ml of spore solution was put into sterile beakers and pre-germinated oat seeds were placed on it. These seeds were soaked in spore solution for 2-3 hours. Each variety was performed in 3 replications. One seed was used for each replication. For the control , one seed was kept in distilled water. Disease assessment was performed on germinated oat seeds 45 days after inoculation according to a modified 0-5 scale (Wildermuth and McNamara, 1994) (Table 2) (Figure 2).

Table 2. Modified 0-5 scale in germinated oat seeds against *Fusarium nygamai.* (Wildermuth and McNamara, 1994; Köycü and Özer, 2019)

Scale Value	Reaction
0	Healthy plant, no color change in root areas
1	Less than 25% area of necrosis
2	Area of necrosis between 25-50%
3	Area of necrosis between 51-75%
4	Area of necrosis greater than 75%
5	The plant is dead

After the disease evaluation, sections were taken from the roots of the plants and reisolation were made.

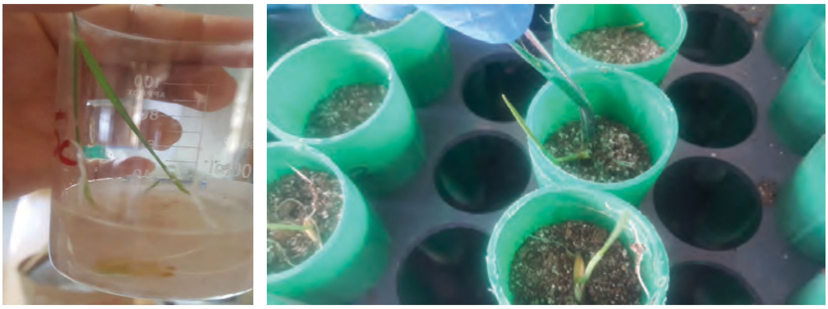

Figure 2. Planting Infected Seed with *F. nygamai*.

Planting sterile seed into infested soil with *F. nygamai*

The sterilized soil, which was soil:peat:sand (1:1:1) mixture, was placed in the sterilized jar. Approximately 15-20 fungal discs were placed in these jars and sterile distilled water was placed in order to provide moisture for the growth of the fungus. These jars were placed in an incubator at 23-24°C and incubated for about 20 days for the spread of the fungus in the soil. Sterile cotton was placed under the pot, and a sterile soil: sand: peat mixture was placed on top. Then, some soil inoculated with *F. nygamai* was added. Oat varieties, which grown for one week, were sown in 3 replications, with one oat seed in each replication of soil infested with *F. nygamai*. Disease assessment of germinated oat seeds on a modified 0-5 scale was performed 45 days after inoculation (Wildermuth and McNamara, 1994). (Table 2) (Figure 3). After disease assessment, reisolation was made from the roots of the plants.

Figure 3. Planting Sterile Seeds into Soil Infested with *F. nygamai*.

Results & discussion

Pathogenicity test

As a result of the pathogenicity test, pathogenicity was evaluated in seven oat cultivars of *Fusarium nygamai* isolate according to the 0-5 scale of Ichielevich-Auster et al. (1985) (Table 3). According to the results of the pathogenicity test, Diriliş variety was susceptible with 100% disease severity among oat varieties, followed by Yazır (53.33%), Katmerli (46.67%), Yeniçeri (46.67%), OTAG (33.33%), Seydişehir (26.67%) and Faikbey (13.33%).

Table 3. The pathogenicity test results of seven varirties of oat inoculated with *Fusarium nygamai*

Variety	Disease Severity Rate(%)	Çeşit	Disease Severity Rate(%)
Diriliş	100	Seydişehir	26,67
Faikbey	13,33	Yazır	53,33
Katmerli	46,67	Yeniçeri	46,67
OTAG	33,33		

Table 4. Disease severity rate(%) and disease mean rate of 'planting infected seed with *F. nygamai*' and 'planting sterile seed to infected soil with *F. nygamai*' studies

Variety	planting infected seed with *F. nygamai*		planting sterile seed to infected soil with *F. nygamai*	
	Disease Severity (%)	Disease Mean	Disease Severity (%)	Disease Mean
Diriliş	46.67	2,33	53,33	2,67
Faikbey	26,67	1,33	26,67	1,67
Katmerli	100,00	5,00	26,67	1,50
OTAG	53,33	2,67	33,33	1,83
Seydişehir	73,33	3,67	26,67	1,67
Yazır	80,00	4,17	46,67	2,50
Yeniçeri	73,33	3,33	40,00	2,00

Reaction studies with planting *F. nygamai* infested seed and planting sterile seed into soil infested with *F. nygamai*

In the study, the disease was evaluated according to the blackish-brown color changes in the oat root according to the modified 0-5 scale (Wildermuth and McNamara, 1994; Köycü and Özer, 2019) in 45 days after inoculation (Table 2). According to the results of the trial of planting seed infested with *F. nygamai* Katmerli cultivar, which had 100% disease severity among the oat cultivars, was the most susceptible oat cultivar. It was followed by Yazır (80.00%), Seydişehir (73.33%) and Yeniçeri (73.33%), OTAG (53.33%), Diriliş (46.67%) and Faikbey (26.67%). It was observed that Faikbey cultivar was the most resistant among these cultivars (Table 4). In terms of disease average, Katmerli oat variety had the highest average (5%) and Faikbey oat variety had the lowest average (1.33%) (Figure 4).

According to the results of planting sterile seed ino soil infecsed with *F. nygamai*', Diriliş variety, which had 53.33% disease severity among the oat cultivars, was the most sensitive oat cultivar among these cultivars. It followed by Yazır (46.67%), Yeniçeri (40.00%), OTAG (33,33%), Seydişehir (26.67%), Katmerli (26.67%) and Faikbey (26.67%) and among these varieties Faikbey, Katmerli and Seydişehir varieties were observed to be the most resistant among these varieties. In terms of disease average, Diriliş oat variety had the highest average (2.67%) and Katmerli oat variety had the lowest average (1.5%) (Table 4 and Figure 4).

As a general result of the study, it was revealed that the Yazır variety, followed by the Yeniçeri variety were susceptible according to the results of planting *F. nygamai* infested seed and planting sterile seed into *F.*

nygamai infested soil. In addition, while the disease severity was found to be lower with 46.67% on Diriliş variety using infested seed, , it was observed that this oat variety was the most sensitive variety (53.33%) among the examined oat varieties whenplanting sterile seed into infested soi. On the other hand, while in the study with infested seed with this fungus on Faikbey variety, the disease severity was found to be lower with 26.67%, the most resistantamong the examined oat varieties.

Planting infected seed with *F. nygamai* Planting sterile seed to infected soil *F. nygamai*

Figure 4. Reaction studies with planting seed infested with *F. nygamai* and planting sterile seed into soil with infested with *F. nygamai.*

In this study, the sequence data of this fungus isolate from NCBI was compared with the isolates of *Fusarium* species in the database by performing BLAST analysis, and it was observed that *F. nygamai* had a 100% similarity rate among *Fusarium* species (Anonymous, 2022). As a result of this study, *F. nygamai* was detected from oat roots for the first time in our country, both morphologically and molecularly.

In this study, Çalışkan et al. (2017) found that while Yeniceri oat variety was resistant to stem rust, Faikbey variety was moderately sensitive; Faikbey variety is moderately susceptible to crown rust disease, Seydişehir variety is medium resistant, and Yeniçeri variety is resistant; While it was seen that Faikbey and Seydişehir cultivars were moderately resistant to powdery mildew disease, but the Yeniçeri cultivar was in the susceptible group. In addition, our study found that whenplanting seed infested with with *F. nygamai* Faikbey was resistant compared to other cultivars, but the Yeniçeri cultivar was between moderately susceptible to moderately resistant group and the Seydişehir cultivar was in the moderately susceptible group. Moreover, our study found that whenplanting sterile seed into soil infested with *F. nygamai*, it was observed that Faikbey cultivar was more resistant to *F. nygamai* compared to other cultivars. In this way, the results of both our study and Çalışkan et al. (2017) study were similar.

As a result, genotypes known to be more resistant to soil-borne pathogens can be cultivated for oat cultivation. It has been concluded that promising genotypes can be evaluated in breeding programs.

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Determination of reactions of some oat cultivars to *Fusarium equiseti* causing by root rot disease

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Summary

Hexaploid oats grown as a cultivated plant (*Avena sativa* L. and *Avena byzantina* Coch.) is an important cereal plant used in both human and animal nutrition. In recent years, the increasing importance of oats in human nutrition in the world and the introduction of its use in industry have led to an increase in production areas. In Türkiye, the importance of oats has increased with the fact that the oat plant has increased economically over the years and has entered the preferred product group with the increase of producer support. Oats can be more susceptible to fungal diseases, especially because it can develop better at relatively low temperatures and high humidity in cool climate cereals and has high water requirement. *Fusarium*, which is the causative agent of root rot, is one of the known pathogens of oats. The disease causes the weakening of the plant along with root rot in plants, which significantly reduces the yield and quality. In this study, seven cultivars of oats (Diriliş, Faikbey, Katmerli, Otag, Seydişehir, Yazır, Yeniçeri) were used and the pathogenicity of *Fusarium* on these cultivars were determined. In the pathogenicity test, Ichielevich-Auster et al. (1985) the 0-5 scale was used. Disease severity of the Diriliş cultivar was found the most susceptible by 86.67%. Studies of the reactions were made with a control and 3 replications by using two different methods. One of the reaction tests seed infested with seed with *Fusarium equiseti*. Another reaction tests was to sow sterile seed into soil infested with *F. equiseti*. The evaluation of root rot with the method of Wildermuth and McNamara (1994) offered a 0-5 scale for disease severity, and was used with the Tawsend-Heuberger formula. According to the results, the reactions of oat cultivars to the pathogen was identified. Disease severity was classifed as below; 0: healthy plant, no color change, 1:less than 25% area of necrosis, 2: area of necrosis between 25-50%, 3: area of necrosis between 51-75%, 4: area of necrosis more than 75%, 5: the plant is dead. Against *F. equiseti* root rot one cultivar (Diriliş) was susceptible and six cultivars (Faikbey, Katmerli, Otag, Seydişehir, Yazır, Yeniçeri) were moderately susceptible.

Keywords: Oat, *Fusarium* root rot, *Fusarium equiseti,* disease reaction

Introduction

In recent years, because of the increase in its importance in human nutrition of oats in the world, the industry has increased in the production area. In the world culture of oats, 2/3 are of white oats (*Avena sativa* L.) and 1/3 are red oat (*Avena byzantina* Koch.) (Forsberg and Reeves 1992).

Compared with other grains, oats need cool, rainy climates and fertile soils with low marginal cultivated areas (Hoffmann 1995). In addition, oats under long-day regimes and short seasons, blossoms and matures quickly. Therefore, in the Nordic countries, oats are an important plant (Buerstmayr et al. 2007). In recent years, the consumption of oats has increased, thanks to the nutritional value of oats in human nutrition (Food and Drug Administration 1997).

Oats (*Avena sativa* L.), is a grain that is used in the world for human nutrition and animal feed. Animal feed is the most extensive use of the oat area. Oats are used as green grass, silage, grain and straw in animal nutrition. Also legume forage crops (forage pea, vetch, etc.) support the plant as it is located in mixtures. Stalks are soft and plentiful leaves are preferred by the animals (Anderson 2000).

In recent years, quite a lot of oats and oatmeal are used at breakfast in the human diet. Oats are rich in protein, in addition quantities of fat, vitamin B1, phosphorus, iron and many minerals (Geçit 2016).

In Türkiye, both dry and irrigated agriculture in the areas has been used for cultivation of oats. The regions where agriculture is made are listed as Central South, Middle North, Marmara, Aegean, Black Sea, Mediterranean, Middle East and North East, respectively. (Geçit 2016).

In Türkiye, forage crops cultivation areas have been increased in recent years, depending on the support of the Ministry of Agriculture and Forestry. While it was 2% in 2000, the oat cultivation area increased to 12% (Sayar 2017).

There were only five registered oat varieties in our country in 2013. In 2020, a significant increase was achieved in the number of oat varieties by registering a total of 22 oat varieties, 6 of which were commercial and 16 belonged to institutions (Faikbey, Seydişehir, Sebat, Yeniçeri, Sarı, Fetih, Kırklar, Kahraman, Haskara, Albatros, Bc Marta, Diriliş, Arslanbey, Küçükyayla, Kehlibar, Kayı, Kupa, Hal kalı, Katmerli, Kazan, Mayas ve Somunyıldızı). (Anon 2013; Anon 2020).

Since oats have low temperature, high humidity and high water requirement in cool climate cereals, they may be more susceptible to fungal diseases. The most important fungal diseases are root rot, rust, powdery mildew and leaf disease. *Fusarium*, which is the causative agent of root rot, is one of the known pathogens of oats. One of them is *F. equiseti*. This pathogen adversely affects yield and quality.

One of the ways to increase the product yield per unit area is the determination and dissemination of high-yielding and high-quality varieties that are suitable for the environmental conditions in which oats can be grown, resistant to root rot diseases. The effects of this disease should be determined in the places where oat production is made in our country and necessary precautions should be taken.

A study was made to determine the contribution of fungi of the genus *Fusarium* to the infection of oat seedlings grown under the conditions of central Poland as well as to determine the pathogenicity of *F. graminearum* to seedlings of several oat genotypes under controlled temperature and humidity conditions. It included 39 oat genotypes. At the six-week seedling stage, the percentage of plants with root and leaf sheath necrosis symptoms was evaluated. In 2010 the percentage of seedlings with disease symptoms ranged from 6.5% to 25%, in 2011 it ranged from 17% to 34.5%, whereas in 2012 from 10% to 25%. In 2010 the disease index ranged from 1.4 to 5.7, in 2011 from 4.5 to 8.8, while in 2012 it was between 2.0 and 5.4 (Kiecana et al. 2013). Xue et al. (2019) have studied nine species from 8800 kernels from 176 oat fields from 2008 to 2017. *Fusarium avenaceum*, *F. equiseti*, *F. graminearum*, *F. poae* and *F. sporotrichioides* were the most commonly isolated species, occurring in 23–66% of the infected fields and 1.3–27.0% of the infected kernels.

Chen et al. (2019) identifed pathogens causing oat root rot. During July and August 2018, severe outbreaks of seedling wilt were observed on oat with an incidence of approximately 5% in the farmland of Shandan County, Zhangye City, Gansu Province. Symptoms included leaf yellowing, plant stunting, rotting of roots, and vascular discoloration in roots. The symptomatic plants were uprooted and collected from several incidence areas in the field.

Methods

Pathogenicity test:

For the pathogenicity test, 7mm diameter fungus was taken from pure *F. equisti* cultures. Each oat cultivar germinated in the incubator was taken separately in 3 replications and put into PDA and *F. equiseti* with a diameter of 7 mm was placed next to it. These petri dishes were placed in a 24-25 °C incubator to develop for a week. At the end of a week, Ichielevich-Auster et al. (1985) was evaluated for pathogenicity using a 0–5 scale.(Table 1)

Table 1. The pathogenicity test- 0–5 scale Ichielevich- Auster et al. (1985)

Scale	Infection type (%)
0	no disease
1	1-10% of the hypocotyl is infected
2	11-30% of the hypocotyl is infected
3	31-50% of the hypocotyl is infected
4	51-80% of the hypocotyl is infected
5	All hypocotyl is infected

The reaction tests on seed infested with *F.equiseti*

4-5 days before fungal inoculation to seed, each cultivar was sterilized separately by immersing it in 5% sodium hypochlorite for 3 minutes and passing it through sterile distilled water 2 times. These sterilized seeds were germinated by placing them in an incubator at 25-27 °C, with 12 hours of daytime/12 hours of night. Then, for seed inoculation, pure *F. equiseti* spore suspension was prepared by adding 20-30 ml of sterile distilled water to the petri plate and removing the spores. The density of the spore suspension was measured with haemocytometer slide as $17x10^5$ spores/ml. Then a few drops of Tween 20 were added to the suspension. The prepared fungal inoculation was carried out by adding 30 ml of the spore solution to the germinated oat seeds separately. Then seeds were soaked in spore suspension for 2-3 hours. Each cultivar was made in 3 replications. One seed was used for each replication. Seed soaked in distilled water was used as a control.

The reaction tests with sterile seed sown in soil infested with *F.equiseti*

The sterilized soil:sand:peat (1:1:1) mixture was placed in a jar and approximately 15-20 fungal discs were placed in it, and very little sterile distilled water was added for the growth of the fungus. The jars were placed in an incubator at 23-24 °C and incubated for 17-18 days for the development of the fungus in the soil. Sterile cotton was placed at the bottom of the pots, sterilized soil:sand:peat mixture was placed on top of it, then soil was placed from jars with fungal growth. Oat cultivars showing one-week growth were sown with 3 replications and 1 oat seed in each replication.

Results and discussion

Disease assessment was performed on a modified 0-5 scale 45 days after inoculation. (Table 2). Against root rot *F. equiseti* one cultivar (Diriliş) was susceptible and six cultivars (Faikbey, Katmerli, Otag, Seydişehir, Yazır, Yeniçeri) were moderately susceptible. This study showed that there are cultivars that can be evaluated in terms of resistance to the diseases.

Table 2. *Fusarium equiseti* for modified 0-5 scale (Wildermuth ve McNamara 1994; Köycü ve Özer 2019)

Skala	Reaction type
0	healthy plant, no color change
1	less than 25% area of necrosis
2	area of necrosis between 25-50%
3	area of necrosis between 51-75%
4	area of necrosis more than 75%
5	the plant is dead

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Determination of the reactions of some maize genotypes to ear rot disease caused by *Fusarium verticillioides*

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Summary

Maize is one of the six cereal plants used to feed the world population. It is among the most produced cereals in the world with its versatile usage area, adaptability and efficiency. The maize plant has many different uses and each part has separate economic value. In addition, maize diseases and fungal diseases of root and crown, stalk and ears are among the most important factors restricting yield. In our country various pathogens are seen in maize fields; including various *Fusarium* species that cause seed, root, stalk and ear rots. This study consisted of 60 genotypes, including different genotypes and some commercial varieties developed by the Maize Breeding Unit of the Maize Research Institute in 2021. The experiment was set up in a randomized block design with 3 replications, with 10 plants for inoculation and 10 plants for control in each replication.

According to the flowering time; before the color of the tassels turned pink and purple *Fusarium* ear rot was establish on the plants by artificial inoculation in order to determine the reactions of the genotypes on 06.08.2021 and 17.08.2021. Each inoculated ear was given 1 ml of inoculum with a syringe, with a density of 5x10^6 spores/ml of spore suspension, and 1 ml of distilled water with syringe to control plants. At the harvest time, the ears were collected by peeling and the disease was evaluated according to the rot and mycelial growth on the grains. The disease severity, which was determined by using a 0-5 scale for ear rot, was calculated according to the Tawsend-Heuberger method. According to the results, the reactions of the genotypes were determined by considering the reaction type scale. If the disease severity was 10% or less it was identified as resistant (R), if it was 11-25% moderately resistant (MR), if it was 26-50% susceptible (S), and if it was more than 50% highly susceptible (HS). Against ear rot disease, 1 genotype (Samada 07) showed R-resistant, 3 genotypes (DKC 6664, Andromeda, Kolosseus) showed MR-moderately resistant, 41 genotypes showed S-susceptible and 15 genotypes showed HS-highly susceptible.

Introduction

The maize plant, *Zea mays* L., is one of the six cereal crops that feeds the world population. It is the most produced grain in the world with its versatile usage area, adaptability and efficiency. The maize plant has many uses and each part has separate economic value. The main usage areas of maize are; fresh consumption (boiling and roasting), canned food, maize flour, starch, chips, snacks, baby-corn grains and animal feed (green parts), oil, sweetener, confectionery, chocolate products, baby foods, salad dressings, alcohol, high fructose maize syrup, toothpaste, ethanol (as an additive to gasoline) and automotive industry, cleaning materials, textile and cosmetics industry. It is estimated that 60% of world maize production is used as animal feed, 20% as human food (direct consumption), 10% as processed food and 10% as seeds with other consumptions (Özcan 2009). Although significant increases in production can be achieved with various methods, there are many factors that lead to significant product losses. Maize diseases and fungal diseases of root and crown, stalk and ears are among the most important factors restricting yield (Miller 1994).

In our country in maize fields; we see various *Fusarium* species that cause seed, root, stalk and ear rots. Although seed spraying is effective for root and crown rot caused by *Fusarium* species, there is no effective control method for the control of stalk, ear and grain rot seen in later periods. For these reasons, these diseases, especially seen in ears and grains, directly affect the yield (Anonymous, 1995). In addition to the loss of yield, in recent years the toxic effect of the mycotoxins created by *Fusarium* spp. in maize grain and products on animals and humans have become important. For this reason, an effective control against mycotoxin-forming disease factors becomes even more important in terms of food safety in maize, which is important for both human and animal nutrition. For this reason, the only management applied in practice in the world is to use resistant varieties (Reid et al., 2002).

It is important that root rot diseases can be controlled in an environmentally friendly and effective management in the long term. Studies have been carried out on *Fusarium* species in maize in the world and in our country, and the most common species in maize was *F. verticillioides*. In maize ears *F. verticillioides*, *F. proliferatum* and some other *Fusarium* species cause the formation of a toxin called fumonisin, which is known to be carcinogenic in humans and other animals.

Fusarium ear and grain rot is shown as the most important disease of maize in Canada, USA and Europe and it is reported that *F. verticillioides* (*moniliforme*) is the most common species in all regions (Miller, 1994). It was determined that ear rot disease was seen at a rate of 58% in 1997 in Aydın and the casual agents causing the disease were *F. moniliforme* and *F. graminearum*. In Çukurova, the disease incidence was found to be 26% (Benlioğlu and Yıldız, 1998; Tatlı, 2002). Reid et al. (1999) reported that cultivating resistant host-plants is effective for the control of ear rot (*Fusarium* spp). In Gürsoy and Biçici (2003), a total of 73 maize samples were examined between 2000-2003 and the *Fusarium* spp. was isolated from 61.5% of the samples. It was reported that the most isolated *Fusarium* species were *F. graminearum*, *F. moniliforme*, *F. oxysporum*, *F. solani* and *F. culmorum*. Demir et al. (2005) found fumonisin B1(FB1) in 52 of 100 samples and fumonisin B2(FB2) in 25 samples in a study samples taken in Samsun and also found that 94% of the samples taken had *F. moniliforme*.

In a study of 20 different genotypes from early and hybrid maize lines in 2003 and 2004, disease severity was found to be 10% in genotypes resistant to Fusarium ear rot in Iran. While KE 76009/1-2-1-2 was the most resistant and KE 77005/1-5-1 was the most susceptible genotype, K SC 500, K SC 302 and K SC 400 K were found to be moderately resistant hybrids (Zamani and Mohseni 2006). In 2003, Uçkun and Yıldız (2007) took 66 samples for Fusarium stalk rot disease in the Southern Marmara Region and the disease rate was found to be 75%. In 2004, 149 samples were taken and they state that the disease rate in the region was 85%, while the disease rate for ear rot was 51% in 2003 and 56% in 2004. The most common cause of stalk and ear rot diseases was determined as *F. moniliforme*, followed by *F. graminearum*. Uçkun (2009) carried out a survey study to determine the status of stalk and ear rot diseases. He reported that while 48 genotypes tested in reaction trials against stalk and ear rot showed a susceptible reaction to stalk rot, 7 genotypes were in the resistant group, 11 genotypes were in the moderately resistant group against ear rot in 2007, 18 genotypes were in the moderately resistant group in 2008.

Ashnagar et al. (2012) investigated the resistance of 14 maize cultivars to ear rot infection against Fusarium maize rot infection (*Fusarium verticilloides*) in their study in Iran. They are divided into resistant, tolerant and susceptible groups. KSC700 and DS499 were evaluated as resistant, Kardura 630 as very susceptible variety. Yıldırım et al. (2016) tested 30 lines with the toothpick method for artificial inoculation of stalk rot and the syringe method against ear rot. As a result, they found 1 line moderately resistant (MR) and 6 lines moderately susceptible (MS) against Fusarium stalk rot. In addition, 12 genotypes were found to be resistant to Fusarium ear rot, and 12 genotypes were found to be moderately resistant.

In our study, in the Marmara region, resistance tests carried out by artificial inoculation against *Fusarium verticillioides*, on important and promising maize material, in order to develop varieties resistant to diseases. In this way, it is possible to reveal the disease resistance properties of the material in question and to select the cultivar candidates from among the resistant materials.

Methods

The main material of the study consisted of 60 genotypes including different genotypes and some commercial varieties developed by the Maize Breeding Unit of the Maize Research Institute in 2021. *Fusarium verticilliodes* isolate, which was determined to be the most common species in the region, was isolated from diseased ear rot samples collected in the previous year for use in reaction studies. After the samples were sterilized, they were plated on PDA and then a single spore was taken from the inoculum and 30 petri dishes containing SNA were incubated for 2-3 weeks. Then, a spore suspension was prepared by adding 20-30 ml of sterile distilled water and removing the spores. The density of the spore suspension was measured with haemocytometer at $5x10^6$ spores/ml. (Jeffers et al., 1994). The prepared spore suspension was injected into the first emerging ear of each plant 7-10 days after the tassel was opened, with a 1 ml syringe (Jeffers, 2003).

At the harvest time, the ears were collected by peeling, the disease was evaluated according to the rot and mycelial growth rates on the grains. The infection type was determined by using a 0-5 scale for ear rot (Jeffers, 2002) (Table 1).

Table 1. Maize of 0-5 scale ear rot (Jeffers 2002)

Scale	Infection type (%)
0	There is no infection, the ear is 100% clean and the infection percentage is 0
1	Infection is 10% or less, infection is limited to a few around the inoculation
2	Infection 11-25%, approximately ¼ of the kernels in each infected ears are infected
3	Infection 26-50%, about half of the kernels on each ear infected
4	Infection 51-75%, more than half of the ear infected
5	Infection 76-100%, almost all of the ears are infected,

Disease severity was calculated according to the Townsend-Heuberger formula, and reaction types of genotypes against ear rot were determined (Zamani and Mohseni 2006) (Table 2).

Table 2. Fusarium Ear Rot Reaction Type in Maize (Zamani and Mohseni 2006)

Reaction type	Disease severity (%)
Resistant (R)	10 or fewer infections
Moderately resistant (MR)	11-25 infections
Susceptible (S)	26-50 infections
Highly susceptible (HS)	More than 50 infections

Results and discussion

According to the flowering times of 60 genotypes before the color of the tassels turned pink and purple Fusarium ear rot was established on the plants by artificial inoculation on 06.08.2021 and 17.08.2021. In order to determine the reactions of the genotypes, one ml of inoculum by a syringe was given to each inoculated ear with a density of 5x10^6 spores/ml of spore suspension, and 1 ml of distilled water by syringe was given to control plants. At the harvest time, the ears were collected by peeling, the disease was evaluated according to the rot and mycelial growth on the grains. The disease severity, which was determined by using a 0-5 scale for ear rot, was calculated according to the Tawsend-Heuberger method. According to the results, the reactions of the genotypes were determined by considering the reaction type scale. If the disease severity was 10% or less it was identified as resistant (R), if it was 11-25% moderately resistant (MR), if it was 26-50% susceptible (S), and if it was more than 50% highly susceptible (HS). One genotype (Samada 07) was R-resistant, three genotypes (DKC 6664, Andromeda, Kolosseus) were MR-moderately resistant, 41 genotypes were S-susceptible, 15 genotypes were HS-highly susceptible to ear rot disease, (Table 3). This study showed that there are genotypes that can be evaluated in breeding programs in terms of resistance to the diseases.

Table 3. Disease severity and reaction type of genotypes in the testing Fusarium ear rot in Sakarya province in 2021

Sequence No.	Test No.	Genotype	Plants with ear inoculation		Control Plants	
			Disease Severity (%)	Reaction Type	Disease Severity (%)	Reaction Type
1	42	Samada 07*	8,11	R	5,86	R
2	**21**	DKC 6664*	15,76	MR	6,36	R
3	**35**	Andromeda*	22,10	MR	7,27	R
4	**11**	Kolosseus*	24,36	MR	4,24	R
5	**36**	Gladius*	35,66	S	1,88	R
6	**9**	Sy Lavaredo*	30,35	S	3,43	R
7	**27**	P1921*	27,88	S	3,64	R
8	**23**	P0900*	25,21	S	4,67	R
9	**48**	ADA 9510*	47,22	S	5,15	R
10	**41**	Sakarya*	31,33	S	5,22	R
11	**37**	P3394*	44,58	S	6,42	R

12	**10**	Sy Fuerza*	35,94	S	6,77	R
13	**6**	PL 712*	48,36	S	6,97	R
14	**8**	Sy Tuscany*	40,00	S	7,27	R
15	**22**	P0729*	39,85	S	7,39	R
16	45	ADASA 16*	36,18	S	7,54	R
17	**12**	Kerubino*	40,61	S	7,58	R
18	**4**	Rodeo*	42,30	S	7,88	R
19	31	P1570*	27,63	S	8,33	R
20	**57**	ZM 20.379	39,65	S	8,62	R
21	**24**	P0937*	31,52	S	9,09	R
22	**28**	P2105*	40,00	S	9,09	R
23	**46**	ADA 523*	49,58	S	10,01	MR
24	**34**	LG30709*	36,58	S	10,03	MR
25	**20**	DKC 6980*	41,27	S	10,80	MR
26	**39**	ADA 334*	41,30	S	11,09	MR
27	**44**	ADA 351*	30,55	S	11,52	MR
28	**58**	ZM 20.372	37,04	S	11,52	MR
29	**38**	LG31545*	42,42	S	11,80	MR
30	**14**	Kontigos*	29,09	S	12,42	MR
31	**47**	Hacıbey*	35,13	S	13,04	MR
32	**2**	Vario*	34,37	S	13,36	MR
33	**56**	ZM 20.367	26,73	S	14,09	MR
34	**15**	Kefrancos*	36,42	S	14,36	MR
35	**5**	AGM 1506*	40,79	S	14,85	MR
36	25	P0573*	42,12	S	14,97	MR
37	**30**	P1574*	27,12	S	15,27	MR
38	**40**	AGA*	32,04	S	15,45	MR
39	43	Pehlivan*	27,50	S	19,91	MR
40	**26**	P0288*	43,28	S	21,46	MR
41	**3**	POL 700*	45,94	S	22,81	MR
42	**52**	ZM 20.407	32,00	S	24,95	MR
43	**55**	ZM 20.469	44,00	S	26,73	S
44	**33**	P32T83*	32,73	S	28,43	S
45	**50**	ZM 20.356	41,15	S	30,30	S
46	**1**	Bambus*	53,33	HS	4,91	R
47	**17**	Macha*	51,52	HS	7,79	R
48	**7**	LG30692*	50,10	HS	8,30	R
49	**32**	P31Y43*	58,79	HS	14,41	MR
50	**29**	P31A34*	51,62	HS	14,79	MR
51	**19**	DKC 6761*	51,41	HS	15,40	MR

52	**16**	KWS 5581*	68,93	HS	16,30	MR
53	**13**	Kilowat*	60,52	HS	19,53	MR
54	**49**	ZM 20.265	50,30	HS	22,17	MR
55	**54**	ZM 20.425	65,76	HS	24,24	MR
56	18	DKC 6442*	61,75	HS	27,07	S
57	**51**	ZM 20.397	53,89	HS	35,70	S
58	**59**	ZM 20.444	77,65	HS	57,76	HS
59	**60**	ZM 20.466	80,52	HS	60,66	HS
60	**53**	ZM 20.413	91,67	HS	62,22	HS

* Commercial varieties included in the testing.

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Identification of soil-borne pathogens in wheat and barley in collaboration with international institutes

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Summary

Wheat and barley are the main sources of nutrients for both humans and animals. Grain yields are annually reduced by common root rot, leaf spot disease (*Bipolaris sorokiniana*, *Fusarium* spp.), and soil parasitic nematodes. Seed pre-sowing treatment with fungicides, nematicides, and agrotechnical measures are effective against these pathogens. On the other hand, the most sustainable technique of plant disease control is the introduction of resistant varieties and the search for new effective donors of disease resistance. With the aim to study an approach to soil pathogen control in wheat and barley, the most important activity is the training of scientific staff. Training should include the isolation of fungi from plant parts, inoculum preparation of fungi (*Fusarium, Bipolaris* species), the Baunacke method and/or paper strip method to extract cysts from the soil, and extraction of vermiform plant-parasitic nematodes from soil. Moreover, we isolated a pure culture of common root rot pathogen, including other fungi and cyst nematodes from Kazakhstan's above-ground parts of wheat and barley above. We sampled 80 wheat samples from three varieties (Steklovidnaya 24, Kazakhstan 10 and 231) and 75 barley samples from 3 varieties (Zhan, Symbat, and Aydin). Each variety has shown a level of resistance to different diseases. In the presence of abiotic factors, cereals are often affected by fungi such as *Fusarium, Bipolaris sorokiniana*, *Alternaria,* and others (Figure 2). The methods were specialized for describing morphological and biological features and isolating individual spores on agar for molecular identification. The molecular properties of the isolates will be studied in the future.

Introduction

Wheat and barley are the main sources of nutrients (Shiferaw et al. 2013), worldwide they are cultivated on 219 million ha, with a yield of 760.9 million tons, and in Kazakhstan 14.3 million tons (FAOSTAT 2022), and 3.83 million tons, respectively (FAOSTAT 2021). However, grain yields are annually reduced by common root rot, leaf spot disease (*Bipolaris sorokiniana*, *Fusarium* spp.), and soil parasitic nematodes (Gholamaliyan et al. 2021) (*Pratylenchus*, *Heterodera, Geocenamus, Ditylenchus, Helicotylenchus, Rotylenchus, Pratylenchoides,* and *Tylenchorhynchus* spp.) Seed pre-sowing treatment with fungicides, nematicides, and agrotechnical measures are effective against these pathogens (Dababat et al. 2018). The most sustainable technique of plant disease control is the introduction of resistant varieties and the search for new effective donors of disease resistance (Khan et al. 2020). To determine a literate approach to soil pathogen control in wheat and barley, the most important activity is the training of scientific staff. In this regard, in June 2022, as part of the intended training of doctoral students in the specialty "Plant Protection and Quarantine" Kazakh National Agrarian Research University conducted a research fellowship at the Transitional Zone Agriculture Research Institute (TZARI) in Eskisehir, Türkiye, in collaboration with the International Wheat and Maize Improvement Centre (CIMMYT). Participants learned methods for the isolation of fungi from plant parts, inoculum preparation of fungus *Fusarium*, *Bipolaris* species, Baunacke method and/or paper strip method for extraction of cysts from the soil, and extraction of vermiform plant-parasitic nematodes from soil.

Methods

Briefly, we evaluated 80 wheat cultivars and 75 barley cultivars in naturally-infested fields in the Almaty region, Kazakh Research Institute of Agriculture and Plant Growing, Almalybak, Almaty Region, Kazakhstan (latitude 43.237589, longitude 76.692629). The climate is continental, marked by cold winters and hot summers. The south is the territory of zones: forest-steppe, steppe, semi-desert, desert, and then foothill and mountain region. The territory presented by various soils: are dark-chestnut, light-chestnut, and chestnut soils. Soils of deserts and semi-deserts are represented by grey soils (Abugaliyeva et al. 2016).

The main aim of this study was to identify varieties colonized by different genera of fungi that are found in different parts of plants and infected by including nematodes. Samples were randomly taken from different fields and varieties of the Almaty region. Plant samples and soils were kept in paper bags labeled with relevant sample information and transported to the laboratory of Eskisehir (Türkiye).

The trials were performed during the spring grain cycle of a 1-year rotation of green manure, soybean, and spring cereal. The seed was planted soon after the soil thawed following the winter freeze. Fields were cultivated by disking after the cereal harvest.

Crowns/stem bases were washed with running tap water to remove soil particles that adhered to the surface and placed on paper towels for drying (Figure 1). Crown and stem base tissues showing symptoms of discoloration were sectioned into pieces (approx. 1-2 cm in length); surface disinfested in 1% sodium hypochlorite solution for 3 min, rinsed three times in sterile distilled water, and plated to Potato Dextrose Agar (Shikur Gebremariam et al. 2018). Cultures were incubated under alternating 12 h of light at 25 °C and darkness at 20 °C for 7 days under cool white and black fluorescent lights (Leslie and Summerell 2006). Cultures were transferred to Spezieller Nährstoffarmer Agar (SNA) and kept for 10-14 days to initiate spore formation. Once spores were formed, conidial suspensions were made in sterile distilled water and transferred to water agar (WA) for single spore isolation (Shikur Gebremariam et al. 2018). *H. avenae* cysts were extracted according to Cobb's decanting and sieving method (Cobb 1918) and by using the Fenwick Can technique (Fenwick 1940). Cysts were collected and surface sterilized with 0.5% NaOCl for 10 min and rinsed several times in distilled water. The cysts were kept in a refrigerator at 4°C for 4 months before being transferred to room temperature (10– 15°C) to induce hatching. The freshly hatched second-stage juveniles (J2) were then used as inoculum in screening tests.

Soils were washed on nested sieves with 850 μm and 250 μm mesh sizes to free cysts from the root system. Cysts from both root and soil extractions were collected on the 250- μm sieve and counted under a stereomicroscope (Cobb 1918).

Figure 1. Parts of roots, sub-crowns, stems of wheat *Triticum aestivum* and barley *Hordeum vulgare.*

Results

At the Transitional Zone Agriculture Research Institute (TZARI) in Eskisehir, Türkiye, in collaboration with the International Wheat and Maize Improvement Center (CIMMYT), we isolated a pure culture of common root rot pathogens, including other fungi and cyst nematodes from above-ground parts of wheat and barley (Kazakh samples). Samples were 80 wheat samples from three varieties (Steklovidnaya 24, Kazakhstan 10, 231) and 75 barley samples from three varieties (Zhan, Symbat, and Aydin). Each variety has resistance to different diseases and pathogens. In the presence of abiotic factors cereals are often affected by fungi such as *Fusarium, Bipolaris sorokiniana*, *Alternaria,* and others. (Figure 2) Methods were mastered for describing morphological and biological features and isolating individual spores on agar for molecular identification. In a further internship, the molecular properties of the isolates will be described using species-specific primers and sequence analysis of the ITS and GPDH loci of genomic DNA.

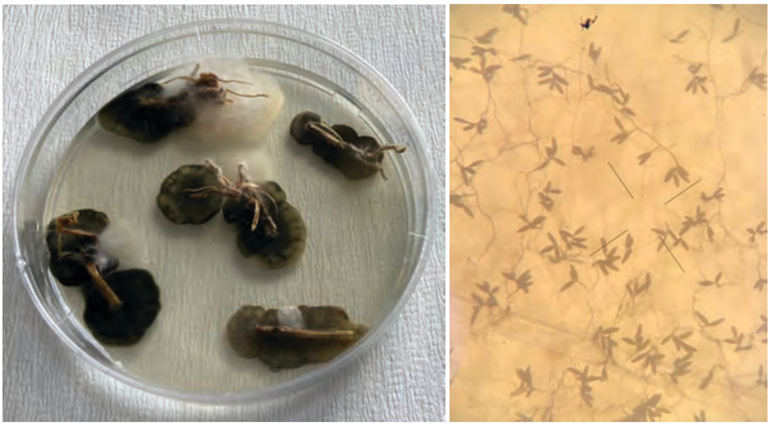

Figure 2. Development of *Bipolaris sorokiniana* Shoem on potato dextrose agar and the conidia under microscopy.

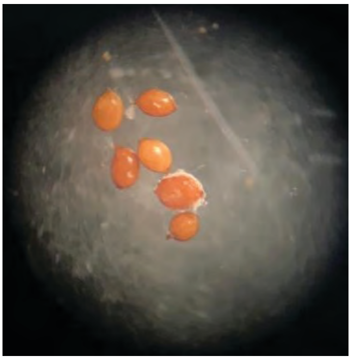

Figure 3. Cyst nematodes *Heterodera filipjevi* under a stereomicroscope.

For nematodes, methods were taught to extract nematodes from soil using various techniques (Cobb decantation and sieving method, use of the Fenwick Can method, and in vitro preparation for nematodes on carrot disks according to Moody et al. (1973)).

Discussion

The purpose of this work was to teach new techniques to control soil pathogens that affect crops, especially wheat and barley. Moreover, cyst nematodes are not far behind in crop damage.

Turkish scientists reported that one of the most effective measures to control these SBPs is the use of host resistance, through which inoculum levels of these pathogens can be reduced to thresholds that cannot cause economic damage. CIMMYT International, in collaboration with the Ministry of Agriculture and Rural Affairs of Türkiye, has developed an international field and laboratory screening program to identify spring and winter wheat samples resistant to SBPs. Several screening protocols for assessing resistance to both cereal root rot and nematodes have been modified and optimized (Nicol et al. 2007).

To keep disease intensity below damaging levels, appropriate management measures, such as crop rotation schemes and the use of resistant germplasms to soil-borne diseases, can be used.

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Influence of crown rot on yield components and trichothecene accumulation in wheat grains

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Summary

Crown rot is an important disease in wheat caused by *Fusarium* species, and it has been spreading in China for several years. The influence of crown rot on yield components and trichothecene accumulation in wheat grains was investigated by collecting naturally infected samples from the coastal area of Lianyungang City, and the yield components and trichothecenes in grains were measured. The trichothecene concentrations in wheat grains with crown rot were compared to those with Fusarium head blight. Artificial inoculation experiments were also carried out, and the trichothecenes in grains were detected. The results showed that the key yield component of wheat affected by crown rot is grain weight, the risk of contamination for wheat grains by DON is small, but the risk of nivalenol (NIV) contamination should not be ignored. Moreover, in the same field, the trichothecenes accumulated in the grains of wheat with Fusarium head blight were many times higher than that with crown rot. The level of trichothecenes in the grains of naturally infected wheat is higher than that in artificially inoculated wheat. The negative effect of crown rot on wheat included not only the yield and quality deduction, but also the risk of food contamination by trichothecenes.

Introduction

In recent years, crown rot has occurred in many wheat-growing areas of China and became a serious issue in the wheat industry (Xu et al. 2018). In China, crown rot of wheat is mainly caused by *F. asiaticum*, *F. graminearum*, and *F. pseudograminearum,* (Deng et al. 2020; Zhang et al. 2015). The yield loss of wheat caused by crown rot and the affected yield components have been assessed. Considering the differences in pathogen species, disease severity, wheat cultivars, environmental and climatic conditions, the yield loss and affected yield components differ between regions and over years (Smiley et al. 2005). An important characteristic of the *Fusarium* species associated with wheat diseases is that they produce a range of mycotoxins that accumulate in crops and threaten the health of humans and animals (Nelson et al. 1994). Among the mycotoxins, type B trichothecenes such as deoxynivalenol (DON), the acetylated derivatives of DON (e.g., 3-ADON and 15-ADON) and nivalenol (NIV), are commonly found in cereal grains and products (Champei et al. 2004; Foroud et al. 2009). DON is a virulence factor and plays an important role in disease development when wheat is infected by *Fusarium* species (Bai et al. 2001; Mudge et al. 2006). The maximum tolerable limits of DON in cereals and cereal-derived products have been set in different regions and countries. In China, the government has set the maximum tolerable limits for DON in cereals and their products to 1.000 μg/kg. The toxic effects of NIV, including immunotoxicity and hematotoxicity, have been determined (Dewa et al. 2011; Kubosaki et al. 2008).

Fusarium head blight (FHB) of wheat causes trichothecene contamination in wheat grain (Foroud et al. 2009). However, whether crown rot of wheat, which is also caused by *Fusarium* species, will expose such a risk of trichothecene contamination in wheat grains should be clarified.

In Jiangsu province of China, the first massive occurrence of crown rot of wheat was observed in the coastal area of Lianyungang City around 2014, and since then, this area has been infested with the disease in successive years and became a typical place of crown rot occurrence in wheat (Deng et al. 2020). By using the samples collected from commercial fields in this area, this study aimed to investigate the influence of crown rot on yield components and trichothecene accumulation in wheat grains.

Methods

In 2017, 250 spikes from disease-free wheat tillers and 250 spikes from crown rot symptomatic wheat tillers (with white head and crown stem base) were collected from a commercial field in Lianyun District, Lianyungang City of Jiangsu Province, which is near the China's Yellow Sea coast. The wheat cultivar was Yannong 19. The incidence of crown rot-diseased tillers was approximately 18%, and the incidence of tillers with FHB was less than 0.5%. In 2018, 250 spikes from healthy wheat tillers, 250 spikes from wheat tillers with crown rot symptoms, and 100 spikes with FHB symptoms were collected from the same field as in 2017, where the wheat cultivar was Lianmai 7, the incidence of crown rot was approximately 11%, and the incidence of FHB was approximately 1.2%. In both years, the samples were collected at GS 87 (hard dough stage) on the Zadoks scale (Zadoks et al. 1974).

Each spike was individually threshed, and its grains were placed into a paper envelope. All the envelopes containing wheat grains were placed in an oven at 60°C for approximately 24 h until constant weight was obtained. The grains were counted and weighed. The difference significance of the yield per spike, grain number per spike, and kernel weight between disease-free and crown rot-diseased wheat were analyzed using two-sample t-test in DPS V 15.10 (Science Press, Beijing, China).

The aggressive *F. asiaticum* isolate CF0915 and wheat cultivar Yangmai 158 were used in artificial infection experiments. The surface-disinfected wheat seeds were germinated for 2 days at 25°C in Petri dishes. For inoculation treatment, the newly germinated seedlings were immersed in the spore suspension with 10^5 conidia per ml for 1 min (Li et al. 2008). For the control, the newly germinated seedlings were immersed in sterile water for 1 min. Five seedlings were planted in a pot 12 cm in diameter containing autoclaved soil. Three replicates, each containing 12 pots, were used. When the wheat grew to GS 87, the incidence of white head was investigated. The spikes from crown rot symptomatic tillers (with white head and crown stem base) in inoculation treatment and from healthy tillers in the control group were collected and threshed. The grains were dried as described above. For the field samples of each cultivar, the grains from the spikes in the same grade were mixed together and ground using a grinder (CT293 CyclotecTM, FOSS Analytical Co., Ltd., Denmark). Trichothecenes were extracted from the wheat flour and purified as previously described (Zhang et al. 2015). The concentration of DON, 3-ADON, 15-ADON, and NIV was analyzed using an LC-MS/MS system equipped with the Agilent 1290 UHPLC, AB Sciex 6500 Qtrap. Three replicates were employed for each sample. The difference significance of trichothecene concentrations between samples was analyzed using one-way ANOVA in DPS V 15.10.

Results

Influence of naturally infected crown rot on the yield per spike of wheat and its components

For the cultivar Yannong 19 investigated in 2017, the grain number per spike of crown rot-diseased tillers was not significantly different from that of disease-free tillers, but the kernel weight and yield per spike of diseased tillers was significantly lower than that of disease-free tillers ($P < 0.05$, Table 1). The same performance was observed from the cultivar Lianmai 7, which was investigated in 2018 (Table 1). Therefore, in the experimental field, crown rot will decrease the yield per spike of wheat, and the key affected yield component is kernel weight.

Table 1. Influence of crown rot on yield per spike and its components of two wheat cultivars

Cultivar	Year	Category	Grain number per spike		Kernel weight		Yield per spike	
			Mean	P value	Mean (g)	P value	Mean (g)	P value
Yannong 19	2017	Disease-free	30.72	0.4108	0.0343	0.0001*	1.0676	0.0001*
		Crown rot-diseased	30.08		0.0189		0.5896	
Lianmai 7	2018	Disease-free	19.93	0.6024	0.0443	0.0001*	0.8989	0.0000*
		Crown rot-diseased	20.27		0.0351		0.7249	

Note: * indicates statistically significant difference.

Influence of naturally infected crown rot on trichothecene accumulation in wheat grains

For Yannong 19, the DON concentration in the grains from disease-free tillers ranged from 50 µg/kg to 61 µg/kg and that in the grains from crown rot-diseased tillers ranged from 97 µg/kg to 166 µg/kg (Fig. 1 A). For Lianmai 7, the DON concentration in the grains from disease-free tillers was 77–133 µg/kg and that in the grains from crown rot-diseased tillers was 223–270 µg/kg. Although the DON concentrations in the grains from crown rot-diseased tillers of the two cultivars were higher than those from disease-free tillers, they were still far lower than 1.000 µg/kg, which is the standard limit of China.

For Yannong 19, the 3-ADON concentration in the grains from disease-free tillers ranged from 16 µg/kg to 19 µg/kg and that in the grains from crown rot-diseased tillers ranged from 14 µg/kg to 16 µg/kg; the 15-ADON concentration in the grains from disease-free tillers ranged from 18 µg/kg to 24 µg/kg and that in the grains from crown rot-diseased tillers ranged from 17 µg/kg to 22 µg/kg. For Lianmai 7, trace or no 3-ADON and 15-ADON was detected in the grains from disease-free and crown rot-diseased tillers.

For Yannong 19, the NIV concentration in the grains from disease-free tillers ranged from 13 µg/kg to 14 µg/kg and that in the grains from crown rot-diseased tillers ranged from 39 µg/kg to 318 µg/kg. For Lianmai 7, the NIV concentration in the grains from disease-free tillers was 10–13 μg/kg, and that in the grains from crown rot-diseased tillers was 213–373 μg/kg. For both cultivars, the NIV concentration in the grains from crown rot-diseased tillers was much higher than those from disease-free tillers.

The concentrations of DON, 3-ADON, 15-ADON, and NIV in the grains from FHB-diseased tillers were 17.733, 400, 167, and 5.600 μg/kg, respectively, which were many times higher than those in the grains from crown rot-diseased tillers.

Influence of artificially infected crown rot on trichothecene accumulation in wheat grains

The concentrations of DON, 3-ADON, 15-ADON, and NIV in the grains from tillers with crown rot in the artificial inoculation treatment were 90, 3, 0, and 10 μg/kg respectively, which were close to those in the grains from disease-free tillers in the control, and no significant difference was observed between the inoculation treatment and the control ($P > 0.05$, Fig. 3).

Discussion

In this study, for two consecutive years, wheat samples collected from a commercial field in the coastal area of Lianyungang City, China were used to investigate the influence of crown rot of wheat on yield components and trichothecene accumulation in wheat grains.

Results show that the yield per spike of wheat was reduced by crown rot, and the root of the reduction lay in the reduced kernel weight rather than the grain number per spike. Therefore, at the sampling site, the crucial period when yield was affected might be grain filling stage. Crown rot of wheat causes the xylem and phloem tissues to be colonized by hyphae 16 weeks after planting (Knight et al. 2016). Moreover, it was also found in our previous observation that, at grain filling stage, the roots of the crown rot-diseased wheat were easily torn away from the stems. Therefore, we infer that crown rot weakens the water conveyance system of wheat. Grain filling stage is the most critical period for water requirement (Liao et al. 2008), and the drought stress during this stage will lead to the decline in grain yield (Lu et al. 2020). We assume that, as a result of the conflict between the high requirement for water and the reduced capacity in water absorption and transportation, the symptoms of premature senescence such as white-head and reduced kernel weight become inevitable when wheat was infected with crown rot.

Several previous studies had explored the mycotoxin contamination in wheat with crown rot. Mudge et al. (2006) and Covarelli et al. (2012) investigated DON accumulation in different sections of wheat plant after inoculating stem bases of wheat with *Fusarium* species. Xu et al. (2021) analyzed mycotoxins such as DON, zearalenone, and deoxynivalenol 3-glucoside in the stems and kernels of wheat that was artificially inoculated with *F. pseudograminearum*. Fan et al. (2021) studied the distribution of DON and its derivatives in field-infected and artificially inoculated wheat plants. Different from these studies, based on sample collection from naturally infected field, the present study focused on the risk of wheat grain being contaminated by trichothecene toxins, and the target toxins included DON and its derivatives, as well as NIV.

The present study showed that naturally infected crown rot would lead to increased DON concentration in wheat grains, but the rise was relatively moderate, and the level of DON was in a safe range. In addition, trace or no derivatives of DON (3-ADON and 15-ADON) were detected in the grains. Therefore, the risk of wheat grains being contaminated by DON caused by crown rot is small, which is consistent with previous finding (Fan et al., 2021). However, for NIV, different situations were observed. In comparison with heathy wheat, much more NIV accumulated in the grains of diseased wheat.

At the experimental location, crown rot and FHB of wheat were caused by almost the same pathogens, namely, *Fusarium asiaticum and Fusarium graminearum*, except that crown rot was partially caused by *Fusarium pseudograminearum* (Deng et al. 2020; Shen et al. 2012). Even so, for the samples collected from the same field, the DON and NIV concentrations in the grains from FHB-diseased tillers were approximately 86 and 23 times of that from crown rot-diseased tillers, respectively.

Notably, in the present study, the concentration of trichothecenes in the grains of naturally infected wheat was higher than that in artificially infected, which was identical with the result of Fan et al. (2021). Fan et al. speculated that the higher level of trichothecenes in the grains of naturally infected wheat might have resulted from the stimulation of mycotoxin production by sublethal dosage of fungicide. It may be a rational explanation, but more detailed and reliable evidence is needed to support this opinion.

Moreover, in the present and previous studies (Fan et al. 2021, Mudge et al. 2006), from the grains of wheat without any symptoms in infected field and in the control in inoculation experiment, a certain amount of trichothecenes was detected. Further research should be carried out to explore the origin of the trichothecenes.

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Identified QTLS conferring resistance to crown rot in wheat by CIMMYT-Türkiye SBP program; recent progress and future prospects

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Introduction

Soilborne pathogens associated with cereals cause significant yield losses throughout the world (Cook 1992). The dryland root rots (foot rot, crown rot) caused by *Fusarium culmorum*, and *F. pseudograminearum* (formerly *F. graminearum* Group 1) are important diseases of cereals around the globe and occur especially under drought conditions in rainfed and wheat monoculture systems. Crown rot (CR), caused by *F. culmorum* and *F. pseudograminearum*, is one of the most destructive diseases of wheat throughout the world. It has been reported in West Asia, North Africa (Egypt, Tunisia, Morocco), the USA, Canada, Australia, and Türkiye (Smiley et al. 2005; Tunali et al. 2008; Chakraborty et al. 2010). Yield losses due to this disease have been recorded up to 35% in the Pacific Northwest (PNW) region of the USA (Smiley et al. 2005) and 25-58% in Australia (Chakraborty et al. 2010). The most important sign of the disease is the browning of the roots, crowns, and stems of the infected plants. Honey brown necrosis can be observed on the leaf sheaths, crowns, and sub-crown internode parts of the plants. Pink-colored hyphal growth can also be seen in plant parts under humid conditions. The disease can also be distinguished in adult plants by the presence of whiteheads (Burgess 2001). Plant infection can occur at all developmental stages starting from germinating seeds to vegetative tissues (Navi and Yang 2008). Drought-stressed plants are more prone to be affected especially during anthesis (Liddell et al. 1986). Management of soil-borne pathogens (SBPs) is quite difficult due to their fast adaptability, survival rates and speed of spreading. Using or breeding resistant crops with high-yield potential is the most efficient and economical way to improve the productivity of the crop and manage soil-borne pathogens, especially in dryland fields. Since 2001, the International Maize and Wheat Improvement Center (CIMMYT)-Türkiye Soil Borne Pathogens (SBPs) program annually screens up to 1000-1500 wheat germplasm accessions against soil-borne pathogens under controlled (growth room, greenhouse) and open field conditions. Many of these germplasms have been identified as promising for resistance to SBPs (Erginbas-Orakci et al. 2013; Erginbas-Orakci et al. 2018). Recent research within the SBP program of CIMMYT has focused on germplasm screening, the potential of this germplasm as a source of resistance, and how to incorporate the new sources of resistance into breeding programs. One of the subtitles in this program is dedicated to CR resistance due to its impact on agricultural productivity. Screening of germplasm, yielded a number of resistant sources, which were reported in the following studies. In these studies, a number of new sources of resistance, and previously unreported QTLs have been identified through association mapping (Table 1). The new sources of resistance to the CR may be useful for selecting parents and deploying resistance into elite germplasm adapted to regions where it is a problem (Dababat et al. 2018). This review reports the QTLs associated with of the most destructive SBPs, CR, in wheat identified by the SBPs program.

Crown rot

Fusarium crown rot (CR) is one of the most destructive soil-borne pathogens worldwide for the wheat. Efforts to control CR are numerous, while finding resistance is a must follow approach. There have been several studies reporting resistance resources against CR. For this purpose, Erginbas-Orakci et al. (2018) evaluated a total of 126 spring bread wheat accessions for CR resistance, which were classified into three groups (resistant to susceptible) according to their CR resistant reactions under growth room, greenhouse, and field conditions. Erginbas-Orakci et al. (2018) reported three marker-trait associations (MTAs) were identified as linked to CR resistance; two of these on chromosome 3B were associated with field crown scores, each explaining 11.4% of the phenotypic variation, and the third MTA on chromosome 2D was associated with greenhouse stem score and explained 11.6% of the phenotypic variation. Other Genome-Wide Association Studies were also carried out for resistance to *Fusarium species*. A set of 189 advanced spring bread wheat lines obtained from the International Maize and Wheat Improvement Center (CIMMYT) were genotyped with 4056 single nucleotide polymorphisms (SNP) markers and screened for crown rot resistance. Eight different markers on chromosomes 1A, 2B, 3A, 4B, 5B, and 7D were associated with Fusarium crown rot resistance (Sohail et al. 2022). In another study done by Pariyar et al. (2020) to identify new sources of resistance to CR and to dissect the complexity of CR resistance, a panel of 161 wheat accessions was phenotyped under growth room (GR) and greenhouse conditions (GH). Analysis of variance showed significant differences in crown rot development among wheat accessions and high heritability of genotype-environment interactions for GR (0.96) and GH (0.91). Mixed linear model analysis revealed seven novel quantitative trait loci (QTLs) linked to *F. culmorum* on chromosomes 2AL, 3AS, 4BS, 5BS, 5DS, 5DL, and 6DS for GR and eight QTLs on chromosomes on 3AS, 3BS, 3DL, 4BS (2), 5BS, 6BS and 6BL for GH.

Management and future prospects

Integrated management such as the combination of biological control, rotation, resistant germplasm, and deployment of QTLs and chemicals are the major strategies to control soil-borne diseases. There have been significant efforts to improve plants' performance against pathogens, however, there is no complete success due to ever-changing and evolving pathogens. Therefore, there is a continuous need for tools and novel approaches to control these diseases. Even though pesticides are the most common and straight forward method to control soil-borne pathogens, one of the environmentally safest ways is the selection or breeding of tolerant or resistant genotypes. Tools of genetics allow us to screen large numbers of germplasm, cultivated varieties, and wild relatives of crops for the mining of novel alleles of the target disease resistance. Quantitative Trait Loci (QTL) and Genome Wide Association (GWAS) are two recent approaches that are utilized for the allele mining of such traits. Discovery and utilization of a resistance gene against CR would therefore be the environmentally safest and most sustainable approach. With this objective in mind, we have been screening a large pool of accessions against CR. Our results revealed that pyramiding two or more QTLs would enhance the performance of resistance (Pariyar et al. 2016). The main focus of the SBPs program is to find new sources of resistance in wheat germplasm and associated QTLs for resistance associated with SBPs. The QTL and marker-trait associations are quite valuable. However, most of them are neither validated nor utilized in breeding programs. There are plenty of results available on QTLs but most of them are still not validated. After validation, the newly identified resistant sources/genotypes could be exploited by breeders to be incorporated into breeding programs. Therefore, identification of marker trait associations or QTLs solves only part of the puzzle. These QTL would have to be validated, gene associated and utilized in the wheat breeding worldwide.

Table 1. Identified QTLs linked to the crown rot and nematodes in wheat through Soil Borne Pathogens Program studies

Trait name	Chr	Associated marker/Haplotype block	Reference
Crown rot (*Fusarium culmorum*)	2AL	Kukri_c57491_156	Pariyar et al. (2020)
	3AS	wsnp_Ra_c16278_24893033	
	3AS	CAP8_c1393_327	
	3BS	CAP12_rep_c3868_270	
	3DL	wsnp_Ex_c14027_21925404	
	4BS	wsnp_Ku_c12399_20037334	
	4BS	tplb0045c06_1675	
	4BS	RAC875_rep_c72961_977	
	5BS	wsnp_Ku_c17875_27051169	
	5BS	Excalibur_c23304_353	
	5DL	Excalibur_c2795_1518	
	5DS	RAC875_rep_c111521_246	
	6BL	BobWhite_c19298_97	
	6BS	RAC875_c17297_341	
	6DS	BS00021881_51	
	2B	549201894	Sohail et al. (2022)
	7D	579535886	
	2B	708689405	
	3A	738043010	
	4B	539004405	
	1A	10214692	
	1A	38715697	
	4B	539004405	
	5B	598755542	
	3B	wPt-2193	Erginbas-Orakci et al. (2018)
	3B	wPt-2766	
	2D	wPt-669517	

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Molecular characterization of *Bipolaris sorokiniana* Sacc. isolates obtained from wheat plants in different agro ecological regions in Türkiye

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Summary

Bipolaris sorokiniana (Sacc.) Shoemaker (telemorph: *Cochliobolus sativus*) is one of the major pathogens that cause disease in cereal plants. High morphological, physiological, and genetic variability makes the fight of this fungus difficult. Fungus causes black point, spot blotch and common root rot and restricts wheat and barley production. The aim of this study is to confirmation of *B. sorokiniana* isolates by species-specific PCR and compare genetic variability based on Internal Transcribed Spacer (ITS) included 5.8S of *B. sorokiniana* isolates from wheat roots in different agro-ecological region of Türkiye. The genetic differences within these isolates have been identified by partial gene sequencing based on the ITS region and established phylogenetic tree.

Introduction

Common fungal diseases in wheat; root and crown, stem and leaf diseases, ear diseases are grouped under three headings. *Fusarium culmorum* (WG Smith) Sacc., *Fusarium pseudograminearum* Aoki and O 'Donnell, *Bipolaris sorokiniana* (Sacc.), *Rhizoctonia cerealis* Van der Hoeven, *Pseudocercosporella herpotrichoides, Gaeumannomyces graminis* var. *tritici* (GGT) (Sacc. Arx and Oliver) is one of the main disease factors of the root and crown rot. *Bipolaris sorokiniana* (telemorph: *Cochliobolus sativus*), is one of the most important fungal diseases that cause spike disease (black point), leaf spot disease (spot blotch) and root rot (common root rot), restricting wheat production (Misra 1973; Aktaş and Bora 1981; Duveiller and Garcia Altamirano 2000).

It is reported that *B. sorokiniana* is one of the most important disease factors in many regions as a factor of leaf spot and root rot in barley and wheat cultivation areas in the world. The agent is common in temperate and subtropical areas all over the world and causes significant economic losses (Nelson and Klime 1962; Duveiller et al. 1998; Saari 1998). It is reported by researchers that if the disease is found in wheat fields, it causes losses from 15% to 100% in wheat production (Mehta 1994; Saari 1998).

In the area of wheat and barley grown in different climate regions in Türkiye a number of different outcomes about the epidemiology and disease severity have been reported, *B. sorokiniana* about the epidemiology and disease severity with different rates have been reported. Aktaş and Bora (1981) reported that *Bipolaris sorokiniana*, which causes disease in the wheat and barley cultivation areas of the Central Anatolia region, constitutes 8.25% disease severity in the region and the loss due to the fungus is 123 kg/ha. According to the study conducted in 1994 and 1995 on disease rates in the Erzurum province, the presence of *B. sorokiniana* in wheat plants was found to be 46.8% in 1994 and 51.1% in barley. In 1995, they determined this rate as 48.9% and 54.5%, respectively (Eken and Demirci 1998).

In a study by Berbee et al (1999) to determine the phylogenetic distribution of virulent plant pathogens within the genus *Cochliobolus*, 65 species and isolates belonging to this genus, partial gene sequences, 54 species and isolate and *gpd* (glyceraldehyde- 3-phosphate dehydrogenase) used the full gene sequence and investigated genetic differences between the species in this genus.Accordingly, in the results of genetic difference analysis based on gene sequences in which many plant pathogenic species including *C. sativus* are included in *Cochliobolus* Group 1, 1.7% difference between the species in the ITS and 5.8S regions and 5.1% in the *gpd* gene region were been reported.

The main objectives of this study are confirmation of *Bipolaris sorokiniana* isolates identified by species-specific PCR conditions and comparing of genetic variability based on ITS region included 5.8S of these isolates from wheat roots in different agro-ecological region of Türkiye.

Methods

Fungal Cultures

Bipolaris sorokiniana isolates from wheat roots different agro-ecology of Türkiye were stored in -80 °C refrigerator within 15% glycerol vials in Plant Mycology Laboratory of Agriculture Faculty of Ondokuz Mayıs University. These were refreshed on 9cm petri dishes include Potato Dextose Agar and morphologically confirmed as *B. sorokiniana* species by Ellis (1971).

Genomic DNA isolation

The single spore isolates of *Bipolaris sorokiniana* were developed on PDA, developing mycelia were collected with a spatula and taken into vials. Mycelium samples were lyophilized with an air vacuum freeze dryer (LABCONCO®) device. The mycelia mass, whose water was change blown away to evaporated blown away and frozen, was crushed with DNA extraction sticks. The mycelium was treated with DNA extraction solution I (Solution I: 200 mM Tris-HCl, 250 mM NaCl, 25 mM EDTA) and after centrifugation for 2 minutes at 2000 rpm, 90 µl of supernatant was transferred to a clean tube with 10 µl extraction solution II (Solution II: 5% SDS). The supernatant was transferred to a clean tube containing 100 µl alcohol (absolute ethanol) and mixed slowly. Then, it was centrifuged for 10 minutes at 2000 rpm and its contents were drained and left to dry overnight. The dried DNA was dissolved in 100 µl of MiliQ water (Ellis et al., 2005) and the amounts of DNA were measured with the help of nano-drop (IMPLENT®) device. Quantities the DNA samples were measured and stored in the freezer at -20°C.

Species-specific PCR

PCR was carried out in a total volume of 25 µl with 10 ng genomic DNA content. The reaction mixture was adjusted to be 1.6 mM dNTP mixture, 0.2 mM (COSA_F / R: 5'-TCAAGCTGACCAAATCACCTTC – 3 '/ 5'-TCTCACCAGCATCTGAATATGA – 3') and 1U Taq polymerase for each primer. In addition, the reaction solution (10X) was prepared by mixing with 75 mM TrisHCl, 20 mM (NH_4) $2SO_4$ and 3 mM $MgCl_2$ (Matusinsky et al., 2010). The annealing temperature was set at 66°C for the first 5 cycles, 64°C for the next 5 cycles, and 62°C for the remaining 30 cycles. While the annealing time was 20 seconds for each cycle, it was determined as 95°C 30 seconds for the first denaturation and 45 seconds for the extension (72°C). In the last extension step, 72°C 5 min. samples were left to cool at 10°C. The PCR products obtained were visualized with 2.5% agarose gel electrophoresis and recorded with the imaging device (BIORAD GEL DOC®).

Amplification of the ITS regions (PCR-ITS)

Genomic DNA from *B. sorokiniana* isolates was amplified using universal primers from fungi designed to amplify ITS1 and ITS2 regions (White et al. 1990). PCR replications were performed with the ITS4 and ITS5 primer pairs of the specified region (ITS4: 5′-TCC TCC GCT TAT TGA TAT GC-3 ′and ITS5: 5′- GGA AGT AAA AGT CGT AAC AAG G-3′). PCR reactions were prepared in a total volume of 50 µl, by mixing 10 ng template DNA, 5 U / µl Taq polymerase, 50 mM, 2 mM dNTPs and 10 × buffer from each primer pair. PCR conditions were 30 cycles at 95 ° C, 30 seconds at 58 ° C and 1 minute at 72 ° C for a total of 25 cycles; It was applied as a final elongation of 10 minutes at 72 ° C (White et al, 1990). 5 µl of the PCR products obtained were visualized with 1.5% agarose gel electrophoresis, the remaining PCR products of the isolates showing approximately 450-500 bp band in the visualizations were purified with the help of the QIA quick PCR Purification Kit (QIAGEN®). 1 µl of the amount obtained was imaged again with the aid of 2.5% agarose gel, and each product belonging to the samples with clear and clear gel images was sent to the company where the reaction sequences prepared for ITS4 and ITS5 primer pairs will be taken.

Phylogenetic Tree

The sequences obtained were sorted and arranged using the Clustal method using Dnastar (Lasergene, Wisconsin, USA) and comparison and verification of the specified gene region of the isolates was made according to the GENEBANK database. All phylogenetic analyzes were performed using PAUP 4.0 b10, Unweighted parsimony analyzes were performed using heuristic options with 100 additional random sequences, individually tree bisection-reonnection (TBR) branching swapping. Kalde constant 1000 bootstrap was evaluated as repetitive. *Alternaria alternata* (KP131535) and *B. spicifera* (JN695636) as outgroups were used in phylogeny.

Results

Thirteen isolates of *B. sorokininana* were identified by morphology of this species on PDA. Except four isolates, totally eight isolates had performed by species-specific primer, COSA F/R, and had positive results. Additionally, all isolates of this species were sequenced by ITS region and amplified totally more than 500bp of this region (Table 1).

A total of nine representative isolates as one or two isolates from wheat plants in each agro-ecological zones of Türkiye and two different species from this species were used for the phylogenetic tree and two main branches included one isolate in a branch and eight isolates in other were obtained by phylogeny of *B. sorokiniana*.

Discussion

This is the first time that a study was carried out on the molecular identification and characterization of soilborne and foliar plant disease caused pathogen *B. sorokiniana* using various representative isolates from wheat production areas of Türkiye. The phylogeny has shown two main groups among these isolates of *B. sorokiniana* (Figure 1). Berbee et al (1999) determined the phylogenetic distribution of virulent plant pathogens within the genus *Cochliobolus* and reported 1.7% differences between the species in the ITS region, which was more diverse than this in *gpd* gene locus. *Bipolaris sorokiniana* is one of the most important soilborne pathogens, therefore in order to understand the pathogenic activity and improve to control strategies of this pathogen, we need more comprehensive efforts related with combined pathogenicity and molecular data, such as multilocus sequencing.

Table 1. The data of *B. sorokininana* locations, pathogenicity, species-specific PCR and ITS sequencing

Fungus&Isolate	**Agro-Ecologic.**		**Mating**	**COSA-F/R**	**ITS**
Name&Code	**Region***	**Pathogenicity****	**Type****	**PCR**	**Sequencing**
Bipolaris sorokiniana B1	Çorum/K.A.B.	*nd.*	-	-	+
Bipolaris sorokiniana B7	Tokat/D.A.B	Pathogen	*MAT*-2	+	+
Bipolaris sorokiniana B10	Samsun/K.B.	Pathogen	*MAT*-2	+	+
Bipolaris sorokiniana B12	Konya/G.A.B.	Pathogen	*MAT*-1	-	+
Bipolaris sorokiniana B15	Yozgat/K.A.B.	Weak Pathogen	*MAT*-2	+	+
Bipolaris sorokiniana B20	Kayseri/G.A.B.	Pathogen	*MAT*-2	+	+
Bipolaris sorokiniana B40	Kastamonu/K.B.	Pathogen	*MAT*-1	+	+
Bipolaris sorokiniana B41	Çankırı/K.A.B.	Pathogen	*MAT*-2	+	+
Bipolaris sorokiniana B52	Şanlıurfa/G.D.A.B	Pathogen	*MAT*-1	+	+
Bipolaris sorokiniana B64	Şanlıurfa/G.D.A.B	*nd.*	-	+	+
Bipolaris sorokiniana B76	Muş/G.D.A.B	Pathogen	*MAT*-1	-	+
Bipolaris sorokiniana B89	Mardin/G.D.A.B	Pathogen	*MAT*-1	-	+
Bipolaris sorokiniana B91	Mardin/G.D.A.B	Pathogen	*MAT*-1	-	+

* KAB= North Anatolian; DAB=East Anatolian; KB= Black Sea; GAB=South Anatolian; GDAB= South-East Anatolian Region

** These data availible from Demir-Sarıaslan (2015) *nd.* not detected

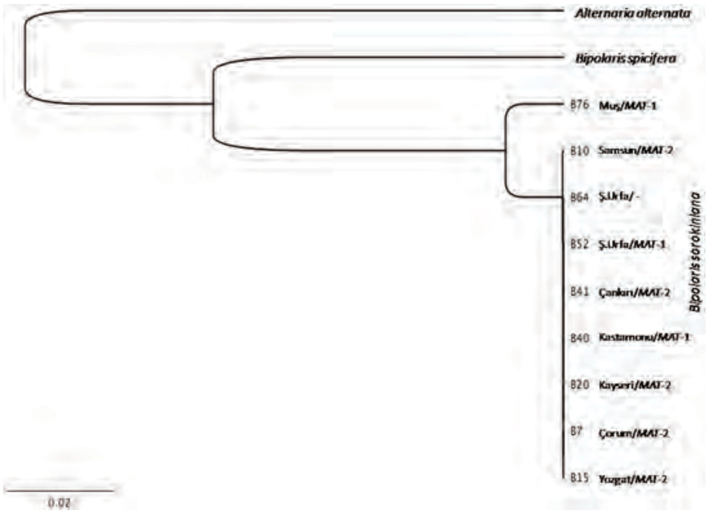

Figure 1. The phylogenetic tree of *Bipolaris sorokiniana* isolates from various agro-ecological zones.

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Session IX
Posters

Efficacy of a Jordanian isolate of *Trichoderma viride* on root knot nematode on wheat

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Summary

The efficacy of a previously characterized isolate of *Trichoderma viride* (*T. viride*-Jo), recovered from the trunk of a date palm grown in the Jordan valley, was studied against root knot nematode (RKN), *Meloidogyne javanica* on wheat. Laboratory experiments showed that *T. viride*-Jo isolate reduced egg hatching of the second stage juveniles (J2s) of the tested RKN. The exposed eggs of the RKN showed deterioration and had many vacuoles. The growth chamber study indicated that pre -sowing seed treatment with *T. viride*-Jo reduced root galling of wheat plants var. Acsad 65 caused by *M. javanica* in comparison to the untreated wheat seeds. Additionally, the pre-sowing seed treatment with *T. viride*-Jo suppressed nematode reproduction and increased feeder roots of wheat plants compared with untreated wheat seeds.

Introduction

The demand for biofertilizers and biocontrol agents is expected to grow to meet the need for the plant-based foods like wheat. The genus *Trichoderma* includes several species that are soil borne fungi with mutualistic endophytic relationships with several plant species. Some species of this fungus have well known antimicrobial and insecticidal activities. Several species of *Trichoderma* were reported to have nematicidal activities (Dababat and Sikora 2006, Jindapunnapat et al. 2013, Naserinasab et al. 2011, Pandey et al. 2003, Sharon et al. 2007). Some strains of *Trichoderma* species were found to be potential biofertilizers (Mahato et al. 2018, Mastouri et al. 2010, Windham et al. 1989). Information on new beneficial traits in biofertilizer and biocontrol strains of species of *Trichoderma* such as the application method and time of application are of great importance. Soil drenching, pre or post planting, is the most published method of application of *Trichoderma*. On the other hand, pre-sowing seed treatment with a pesticide is a well-established practice for most agricultural crops. This treatment is widely practiced to protect the crop from seed and soil-borne diseases. In this context we aimed in this study to evaluate pre-sowing wheat seed treatment with a local Jordanian isolate of the fungus *T. viride*-Jo to enhance growth and suppress root knot nematode (RKN), *M. javanica*.

Methods

The RKN was isolated from infested roots of cucumber grown in uplands area in Jordan. The isolate was identified as *M. javanica* based on morphological characters (Hartman and Sasser 1985, Jepson 1987, Nickle 1991). Egg masses were handpicked and used as the nematode inocula in the different assays. A local isolate of the fungus *T. viride*- Jo recovered from the fungal growth on the trunk of a date palm tree grown in an orchard in Wadi Al- Rayyan/Jordan Valley was used in this study. The fungus *T. viride* was characterized morphologically, enzymatically, and molecularly (Abu Zahra unpublished). This fungus also showed some insecticidal and fungicidal activities (Abu Zahra unpublished).

The efficacy of the fungus *T. viride* on hatching of second stage juveniles (J2s) of *M. javanica* was evaluated *in vitro*. A disc of *T. viride* was excised from a pure culture petri dish and placed on a new potato dextrose agar (PDA) petri dish. A total of three eggmasses of the RKN *M. javanica* were placed around the fungal disc. PDA petri dishes having only RKN eggmasses or fungal discs served as check controls. Each treatment was replicated three times. Petri dishes were checked periodically to monitor the growth of the fungus and the hatched J2s of the RKN (*M. javanica*). Numbers of hatched J2s were recorded after one week.

A growth chamber experiment was conducted on 12 cm open tubes to investigate the pre-sowing seed treatment with the fungus *T. viride*. The spore suspension of the studied strain of *Trichoderma* spp. was prepared following the method described by Xue et al. (2017). A 10-day old pure culture of *T. viride*-Jo grown on PDA was washed with sterile distilled water containing 0.01% Tween 20, then the conidia were dislodged by scrapping using a sterile microscope slide. Finally, the scraped conidia were filtered through two layers of cheesecloth. The concentration of the resulting conidia suspensions was determined using a haemocytometer. Wheat seeds of the cultivar Acsad 65 were treated with a conidia suspension of *T. viride* strain containing 10^7 conidia/ ml at the rate of 5.0 ml/ kg seeds (Xue et al. 2017). Seeds were treated with the fungal conidia suspension in a 250-ml Erlenmeyer flask and shaken to insure uniform coverage of the seeds. Treated seeds were air dried overnight until used. Treated seeds were sown in a 12 cm open tubes filled with soil. A total of 5 egg masses were added to each tube. Treated seeds without nematodes, untreated seeds with or without nematodes were used as controls. Each treatment was replicated five times. Tubes were checked periodically to monitor the growth of the wheat and harvested after one month of planting. Plant parameters including plant height, foliage weight, root weight, and root galling were documented and tabulated.

Results and discussion

The *In vitro* assay showed that hatching of J2s of RKN *M. javanica* was greatly reduced (80%) when egg masses were exposed to the fungus *T. viride* compared to non-exposed egg masses. Moreover, the exposed eggs had many vacuoles while the control eggs did not have vacuoles and mostly, they hatched.

Pre -sowing seed treatment of wheat with *T. viride*-Jo resulted in enhancing the growth of the feeder roots compared to the untreated seeds; however, it did not enhance the plant height and foliage weight over the control (Table 1). Mahato et al. (2018) showed that the soil drenching with *T. viride* ($2x10^7$ conidia/ml) enhanced some wheat plant parameters (plant height, root weight, leaf length, panicle weight, number of grains, grain yield, biological yield, and biomass yield) and had negative effects on other parameters (root length, number of leaves, tiller number, panicle number, panicle length). However, they found that the wheat growth and yield parameter showed the highest value when this fungus and NPK fertilizers were accompanied with farmyard manure.

The wheat seeds that were inoculated with 5 egg masses of the RKN *M. javaica* did not succeed to reach the seedling stage and both seeds and roots were deteriorated. It is well known that high initial population of RKN can kill its host plants, especially when these plants have small roots. Interestingly, when wheat seeds were treated with the fungus *T. viride* before sowing and inoculated with RKN they succeeded to the seedlings stage and roots had few galls (Table 1). Previous reports indicated that the soil drenching with the fungus *T. viride* reduced root galling of many crops (Dababat and Sikora 2006, Pandey et al. 2003). Up to our knowledge, this is the first report of investigating the efficacy of pre-sowing wheat seed treatment with the conidia of *Trichoderma* to suppress the effect of RKN. Xue et al. (2017) reported that seed treatment of spring wheat with a novel strain of *T. harzianum* significantly improved emergence, dry weight, yield of wheat, and reduced root rot severity.

Our findings are preliminary and more laboratory experiments are needed to optimize the process of using pre-sowing seed treatment with *T. viride*. Further field trials have to be done to confirm the results of our laboratory and growth chamber experiments and to prove that this isolate of *T. viride* can be used as a biocontrol agent and as a biofertilizer.

Table 1. Effect of pre-sowing wheat seed treatment with *Trichoderma viride* on wheat root galling, foliage and roots after four weeks of planting

Treatment	Plant height cm	Foliage weight gm	Root weight gm	No. of galls
Seeds without treatments	21	0.2	0.19	-
Pre-sowing seed treatment alone	21	0.2	0.31	-
RKN alone	0	0	deteriorated	deteriorated
Pre-sowing seed treatment and RKN	19	0.34	0.11	5

Data are averages of 5 replicates

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Evaluation of Fusarium crown rot disease (*Fusarium culmorum*) resistance in Jordanian durum wheat landraces

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Fusarium crown rot (FCR) disease, caused by a complex of *Fusarium* species, is one of the major diseases affecting wheat production worldwide and particularly in dry areas. Durum wheat (*Triticum turgidum* L. ssp. *durum*) production suffer significant yield losses due to FCR. Nowadays, climate change plays an important role in increasing disease severity and aggressiveness of FCR that is associated with drought and hot conditions. Utilization of durum wheat landraces in breeding programs is considered a promising strategy to identify new sources of resistance against FCR. Therefore, the main objectives of this study were to evaluate the response of selected Jordanian durum wheat landraces against *F. culmorum* infection under controlled and field conditions and to identify new genetic resources for resistance against *F. culmorum* in a selected set of Jordanian durum wheat landraces. For this purpose, a set of 24 Jordan durum wheat landraces with two checks (with known resistant reactions: moderate and susceptible) were evaluated for their responses to *F. culmorum* infection under controlled conditions (germination stage by using Petri-dish test and seedling inoculation in greenhouse experiments by using three colonized grains and colonized grains- soil mix) and under natural infested field conditions. Different parameters were considered at germination stage that included germination percentage (G%), germination rate reduction (GR), coleoptile length (CL), coleoptile length reduction (CLR), radical length (RL), number of seminal roots (SRN), longest seminal root (LSR) and the Standardized Area Under Disease Progress Curve ($AUDPC_{standard}$). At seedling stage germination percentage (G%), FCR score, seedling height (SH), and disease index (DSI) were evaluated. For field trials, plant height (PH), peduncle length (PL), total weight (TW), grain weight (GW), spike number (SN/ m2), spike weight (SW), spike length (SL), spikelet number, grains per spike (G/S), and kernel characteristics and FCR score, and disease index (DSI) were recorded. At the germination stage, *F. culmorum* had negative effects on most of studied parameters. The $AUDPC_{standard}$ parameter showed that 14 out of 25 genotypes were found to be tolerant, four moderately susceptible and seven were highly susceptible. Using the three colonized grains assay at the seedling stage, only four genotypes were found to have moderate tolerant against FCR, while seven genotypes had moderate tolerant when the colonized grains soil mix assay was used. Results of the genotypes reaction under natural infected field identified a wide range of variability and effect of *Fusarium* on agronomic traits at adult stage that varied among genotypes. Out of the nine genotypes tested including the two checks, two genotypes were found to be moderately tolerant and *Fusarium* infection had a little effect on agronomic traits especially on total kernel weight (TKW). The reaction of these two genotypes were semi-consistent at the three stages (germination stage, seedling, and adult). Therefore, these two genotypes could be utilized to develop new FCR-resistant genotypes in durum wheat breeding programs and for the mapping of quantitative trait loci (QTL) associated with FCR resistance.

Occurrence and distribution of rice root-knot nematode in rice-wheat cropping region of Punjab and assessment of host resistance

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Introdcution

Rice root knot nematode *Meloidogyne graminicola* is a significant pathogen of rice and has spread in South and South East Asia. *M. graminicola* has been equally distributed in different rice cultivated regions of the globe (Fig. 1A) (Bridge et al. 2005). This nematode causes typical hook-like galls at the tip of the roots, chlorosis and lowers the number of tillers and eventually prompts stunted development, empty panicles and low paddy yield (Rahman and Taylor, 1983). Root knot nematode *M. graminicola* is predominant in the United States, India, Bangladesh, Vietnam and Thailand (Yik and Birchfield 1977). In Pakistan, *M. graminicola* was first time found to be associated with rice roots from Sheikhupura in 2003 (Munir et al. 2003). Recently, it was reported in Pakistan as an emerging threat to rice and wheat cultivation because if effects both these crops (Jabbar et al. 2015; 2021). The consequences of the prevalence of this nematode could be more amplifying in the regions where wheat is sown after rice i.e. rice-wheat cropping pattern. Wheat is the staple food for the people of Pakistan and the national food security program is largely dependent on wheat production (Ali et al. 2015). Similarly, rice is one of the main agricultural commodities which are not only highly consumed at the national level but also exported to earn substantial foreign exchange. By keeping the importance and impact of these crops in national scenario, the emerging challenge of *M. graminicola* must be addressed by studying its prevalence, distribution and investigation of resistance level of local rice and wheat germplasm.

Distribution and prevalence in wheat-rice cropping region

Three districts in the central Punjab, Pakistan namely Faisalabad, Hafizabad and Sheikhupura which particularly follow wheat-rice cropping pattern were surveyed for the distribution and prevalence of *M. graminicola* in rice. Fig. 1B represents the location of these three districts in the map of Pakistan in Punjab. Random sampling was done and samples were collected from different locations from each district during the cropping season of 2017. From each field, five root samples of rice plants were dug out with the help of spade/digger with complete rhizosphere and 1000ml composite soil samples were collected in polythene bags after proper labeling from the infection site. These samples were transferred in Plant Nematology laboratory in the Department of Plant Pathology, University of Agriculture Faisalabad for further processing. Additionally, the root samples of some weeds and sorghum showing hook-like galls were also collected. Roots were washed gently with running water to remove soil and plant debris. Identification of *M. graminicola* was carried out on the basis of perineal pattern of female nematodes (Chitwood 1949; Taylor et al. 1955). Nematode prevalence was determined by using following formula according to Norton 1978.

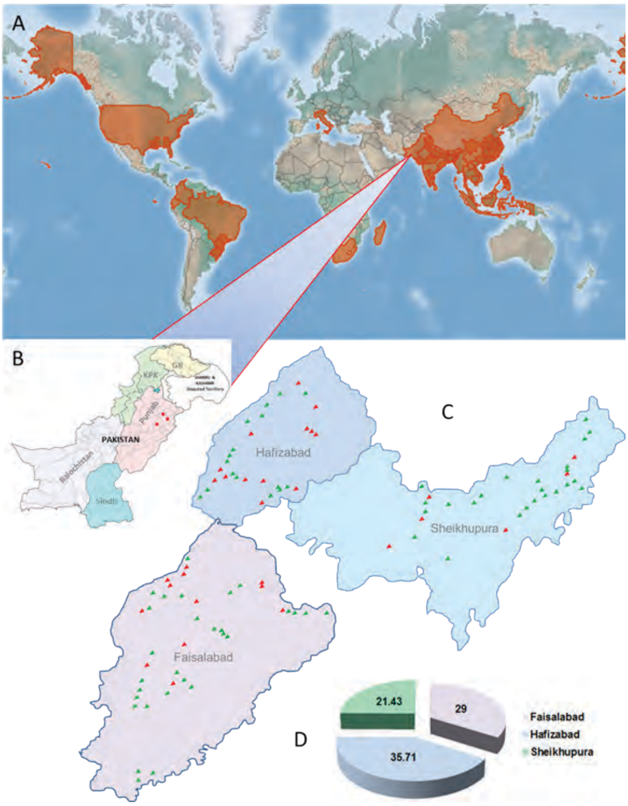

Figure 1. Distribution of *Meloidogyne graminicola*,
A: Global distribution, the countries shaded with red colour exhibit the occurrence of *M. graminicola* (Source: CABI 2022),
B: Map of Pakistan showing the districts under study with wheat-rice cropping pattern,
C: The locations surveyed in different districts, green arrow shows absence of *M. graminicola* which the red arrow displays presence of the nematode, **D**: Percentage prevalence of *M. graminicola* in different districts under study.

Percentage prevalence=(Number of fields infected with root knot nematodes)/(Total number of fields surveyed)×100

The locations are marked on the map of these 3 districts by using latitude-longitude coordinates. On the map given in Fig. 1C, the green arrows point the location with no incidence of *M. graminicola* whereas the red arrows demonstrate the coordinates of locations with nematode infection. The prevalence results from the survey of these 3 districts revealed the distribution of *M. graminicola* in all the districts. The percentage prevalence was maximum in Hafizabad district (35.71%) followed by Faisalabad (29%) and Sheikhupura (21.43%) (Fig. 1D). Recently, in the districts of Chiniot and Faisalabad, we have reported 20 and 27.5% prevalence respectively and these results were supported by molecular identification of *M. graminicola* (Jabbar et al. 2021).

Symtoms, alternate hosts and morphological characterization

The samples collected during the survey from the rice fields displayed clear hook-like knots which are the characteristic of *M. graminicola* (Fig. 2A). Similar symptoms were observed in the weeds present in the rice fields and younger maize plants adjacent to the rice fields (Fig. 2B-E).

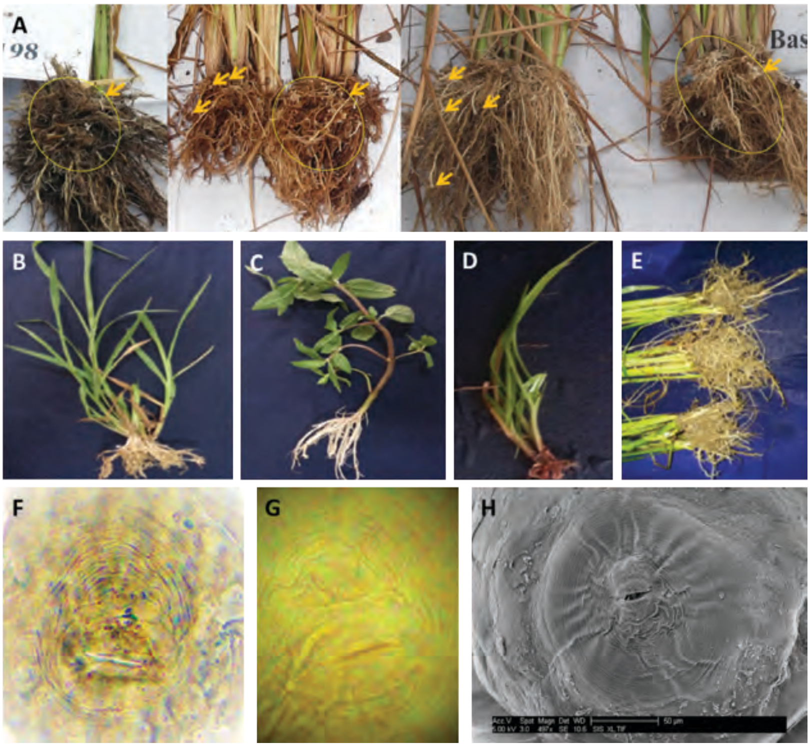

Figure 2. Root symptoms with typical hooked knots (**A**), alternate hosts (**B-E**) and morphological characterization of *M. graminicola* on the basis of perineal patterns (**F-G**). SEM imaging of the perineal pattern (**H**).

The alternate hosts included barnyard grass (*Echinochloa crusgalli*), false daisy (*Eclipta alba*), della grass (*Cyperus rotundus*), and maize (*Zea mays*). It is reported that the nematodes perpetuate to the next crop season through alternate hosts which act as significant reservoir of nematodes (Queneherve et al. 1995). The roots were used for extraction of females to study perineal patterns. For this purpose, the hooked gall tissue was teased apart with the help of forceps or dissecting needle to isolate the adult females. Perineal patterns of mature female nematode were excised and fixed according to the standard method following Jepson (1987). Several perineal patterns were examined under compound microscope (Meiji Techno TC 5200) at 40X for identification (2F&G). External view of the female root knot nematode perineal pattern was graphed under scanning electron microscope (SEM) in Brigham Young University, Provo Utah, United States of America (2H). The perineal patterns clearly demonstrated the identification of these isolates as *M. graminicola* (Yik and Birchfield 1978; Bernard and Eisenback 1997).

Assessment of host resistance in rice and wheat

Resistant cultivars could be an important intervention to manage rice root-knot nematodes in rice and wheat. Sixty rice germplasm accessions were assessed for resistance to *M. graminicola* using sick plot technique. The sick plot was developed at the department of Plant Pathology, University of Agriculture, Faisalabad by planting highly infested nursery of highly susceptible variety 'Basmati 385' for four consecutive years to develop high pressure of the nematode inoculum. Twelve cultivars and forty eight advanced lines of rice were evaluated. Forty wheat varieties were assessed in the Rabi season of 2018-2019. Each variety/cultivar was grown in micro plots in three replicates with twenty plants of each variety for each micro plot. Experiment was laid out under Randomized Complete Block Design (RCBD). The infestation level of *M. graminicola* in field was 310 nematodes/100cm^3 of soil before transplantation. The reaction of rice and wheat cultivars/advance lines were assessed through modified root-galling severity index (Srivastava et al. 2011) also mapped in Fig. 3C. The data were recorded grain weight per plant maturity.

The radar diagrams shown in Fig. 3A and 3B demonstrate the rice germplasm resistance responses and grain weight per plant. Sufficient diversity was observed in the rice accessions for nematode resistance and grain yield. Different categories of rice germplasm according to the Srivastava et al. (2011) disease rating scale are presented in Fig. 3C which shows that 10 accessions indicated tolerant response, 18 partially tolerant, 18 moderately susceptible, 13 susceptible and one demonstrated highly susceptible response to *M. graminicola*. None of the rice genotype showed resistant response. Rice advance lines including K-54, Ph-255, Ph-259, K-69, Ph-101, Ph-225, K-65, Ph-103, K-74 and K-77 showed tolerant response whereas among the partially tolerant 18 genotypes, varieties like NIAB IR-9, Basmati 515, Basmati-2000 and Basmati-198 were included which could be recommended for rice cultivation.

However, rest of the rice accessions were tending toward the susceptible range. It was reported that all rice cultivars of Nepal are vulnerable to *M. graminicola* and insert a constant yield loss danger in rice nurseries and fields (Pokharel et al. 2007). Soriano et al. (2000) and Rao (1985) reported that few IRRI varieties are found to be resistant to *M. graminicola* and proper management with the help of non-host crop rotation significantly decreases its population. Very strong negative correlation was observed between number of galls and grain yield per plant (Fig. 3D). This obviously suggests that increasing number of galls will lead to reduced grain weight in rice.

The wheat germplasm used for resistance study against *M. graminicola* did not demonstrate substantial diversity that is quite obvious from radar and bar diagrams given in Fig. 3 E & F. Only three wheat cultivars namely Galaxy 2013, Faisalabad-2008, and Shafaq-2006, presented partially tolerant response to rice root knot nematode. Importance of tolerant and partially tolerant wheat germplasm for neamtode resistance has been advocated in the literature (Ali et al. 2019). None of the germplasm accessions exhibited tolerant or resistance response in wheat. These results clearly articulate the dire need for the development of rice and wheat cultivars with significant degree of resistance that they can with stand the *M. graminicola* challenge in addition to culmination of grain yield losses imposed by this nematode specie in the wheat-rice cultivation zone of Punjab, Pakistan.

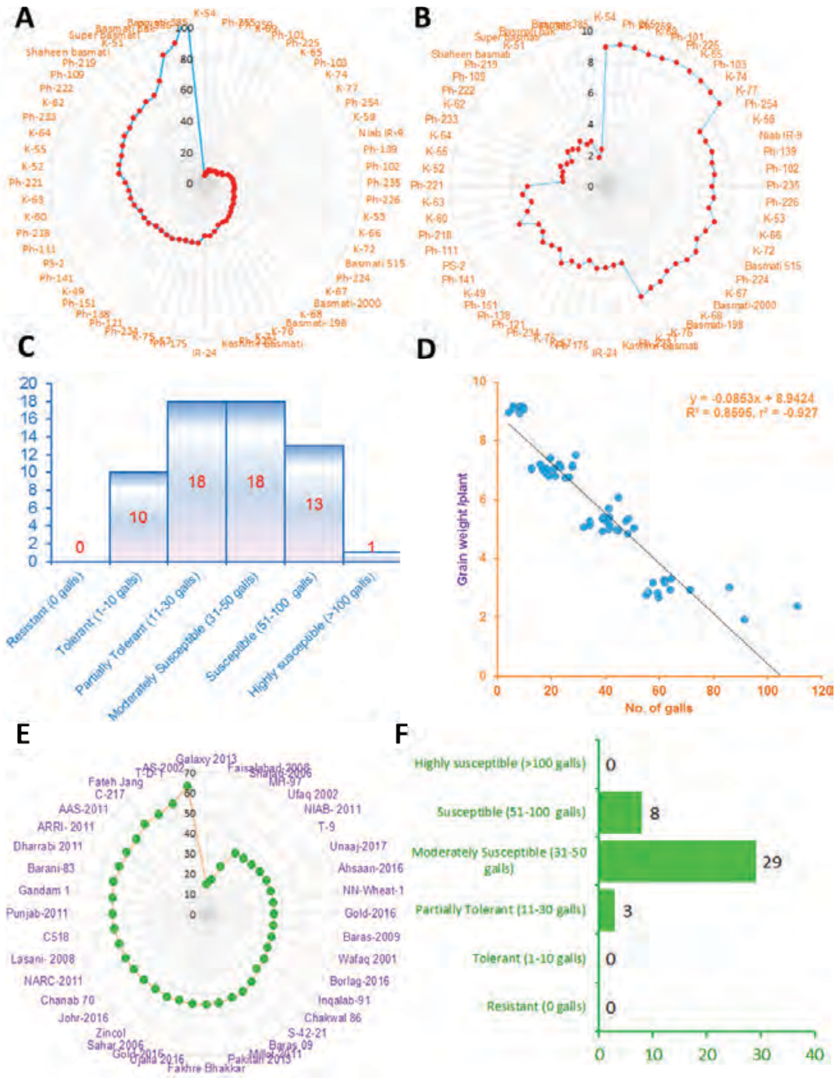

Figure 3. Performance of different rice germplasm for number of galls (**A**) and grain weight per plant (**B**), resistance response groups of rice germplasm against *M. graminicola* (**C**), scatter plot and trend of grain weight in response to number of galls in rice genoptypes (**D**), Performance of different wheat germplasm for number of galls (**E**) and resistance response groups of wheat varieties to *M. graminicola* (**F**).

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Resistance sources in evaluated wheat cultivars and lines to *Pratylenchus thornei* and *P. neglectus*

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Summary

Root lesion nematodes, RLN (*Pratylenchus* spp.) are one of the important parasitic nematodes of cereals worldwide. *Pratylenchus neglectus* and *P. thornei* are the most common species of root lesion nematodes in 38% of wheat and barley fields in Khuzestan province. Regarding importance of using resistant wheat cultivars as an environmentally friendly method for reduction of crop losses caused by RLN, 53 wheat cultivars and lines from Khuzestan province were tested against root lesion nematodes. For this purpose, germinated wheat seeds were planted inside a 3 cm diameter polyethylene pipe filled with 100 cm^3 sterilized soil. One week later each pot received one ml of a suspension containing 400 of different developmental stages of RLN, either *P. neglectus* or *P. thornei*. This experiment was arranged in a completely randomized design in 7 replications maintained 9 weeks at greenhouse conditions at a temperature of 25℃ with alternating of 12 hours of light and darkness. The results showed that 11, 10, and 32 cultivars and lines of wheat were susceptible, moderately resistant, and resistant to *P. thornei*, respectively, however similar results were found for *P. neglectus* by 3, 10, and 40 cultivars and lines with susceptible, moderately resistant, and resistant reactions respectively. In conclusion out of the wheat genotypes tested, 27 wheat cultivars and lines had resistant reaction to both species which contained seven wheat cultivars namely Behrang, Dena, Karkheh, Shush, Star, Yavarus, Setareh and 20 lines which is further discussed. The results of this research provide promising prospects for utilizing these sources of resistance in wheat improving program.

Introduction

Cereals are one of the most important sources of food for humans in the world. Wheat, corn, and rice have a large share of the annual cultivated area (about 58%) among cereals and also supply about 50% of the calorie's humans need. The world's population will reach 8 billion people by 2030, and the food need for cereals will increase with the growing world population (Fischer et al., 2009). Wheat is known as the most important strategic product in Iran. The cultivated area of 4.5 million hectares and the production amount of 13.3 million tons is reported in the crop year 2017-2018. Khuzestan province ranks first and second in Iran regarding production and cultivated area, respectively (Ahmadi et al., 2019).

Cereal cyst nematodes (*Heterodera* spp.) and root lesion nematodes, *P. neglectus* and *P. thornei* are widely distributed in cereal fields in Khuzestan Province southwestern part of Iran. The findings show that root lesion nematodes in 38% of wheat fields in Omidiyeh, Andimeshk, Ahvaz, Baghmalek, Behbahan, Dezful, Ramshir, Ramhormoz, Shadeghan, Shush, Shushtar, Gotvand, Lali, Masjed Soleyman and Hoveyzeh. The average population density in these areas was determined (1-365) 39 nematodes/ gram of root and (1-1334) 101 nematodes /250 cm^3 of soil (Ahmadi *et al*., 2015). The common species of root lesion nematodes in addition to causing direct damage to wheat plants, provide a suitable conditions for the activity of other fungal pathogens involved in root rot. One of the most important management methods to control these nematodes is the use of resistant and tolerant wheat cultivars. For this purpose, initial works were started in Khuzestan province. The goals of this study were to investigate the response of wheat cultivars and lines to root lesion nematodes to obtain adequate information on management methods to prevent yield reduction.

Methods

Seeds preparation and cultivation of wheat genotypes: In this research, 53 wheat cultivars and lines from Khuzestan province (Table 1) were prepared along with GS50a and Warigal as the relatively resistant and susceptible control lines for *P. thornei* respectively, while VP5053 and Trident were included as relatively resistant and susceptible control lines respectively for *P. neglectus*. After preparing the required seeds, they were treated with 2% sodium hypochlorite solution and sterilized 2 times at 5 minutes intervals in distilled water. Their germination was done on moist paper in a petri dish, and germinated wheat seeds were planted inside polyethylene pipes 3 cm in diameter. These pipes contained sterile soil composed of one part sand, 29 parts farm soil, and 70 parts organic matter.

Preparation of inoculum of root lesion nematodes: Soil and root samples containing root lesion nematodes were collected from the wheat fields. The gravid females of *P. neglectus* and *P. thornei* extracted from the samples were then cultured on carrot discs (Muddy et al., 1973). After successfully propagation the nematodes were extracted and used as inoculum for the experiments.

Inoculum procedures: One week after planting the seeds, each of the experimental units in 7 repetitions was inoculated with one ml of a suspension contained 400 of different developmental stages of RLN, either *P. neglectus* or *P. thornei*. This experiment was arranged in a completely randomized design in 7 replications maintained 9 weeks at greenhouse conditions at a temperature of 25℃ with alternating of 12 hours of light and darkness.

Plants harvesting: The plants were removed from the soil after 9 weeks of inoculation. Then, the nematodes from root and soil were isolated using the tray method. Counting the nematode population in soil and roots was done three times separately for two species in a volume of one ml using microscopic slides.

Calculating nematode reproduction factor (Rf) and data analysis: Reproduction factor was calculated for all the cultivars and lines by the final nematode population (Pf) which consists of the sum of nematodes in the soil and root for each line/cultivar divided by the initial population (Pi=400). This experiment was repeated twice. Finally, the tested lines and cultivars were statistically grouped (Toktay et al., 2015).

Results

The results showed that 11, 10, and 32 cultivars and lines of wheat were susceptible, moderately resistant, and resistant to *P. thornei*, respectively. The wheat cultivar Dena and S-78-11 and SS-85-20 lines had the lowest Rf and the highest Rf was observed in the susceptible control cultivar (Warigal) and Chamran with 5.3 and 6.6 respectively. Also, there were similar results for *P. neglectus* species, 3, 10, and 40 cultivars and wheat lines showed susceptible, moderately resistant, and resistant reactions to this species, respectively. The D-84-9, D-85-17, D-86-13, and 2009-6-3 lines, had the lowest Rf while the highest value was observed in susceptible control (Trident) and Virinak cultivar by 4.3 and 2.2, respectively (Table 1). The findings indicated that 27 wheat cultivars and lines including's, Behrang, Dena, Karkheh, Shush, Star, Yavarus, Setareh, D-85-15, D-86-13, D-82-6, D-83-1, D-86-16, D-85-17, D-87-3, D-83-8, D-87-15, SD-84-8, S-80-18, S-87-20, S-87-22, S-84-14, S-91-13, S-92-19, SS-85-20, SS-85-11, SS-85-6 and 514 were resistant to both nematode species. Twenty-three wheat cultivars and lines had different responses to both species, so that their response was susceptible to one species and resistant to the other. For example, the Chamran cultivar was susceptible to *P. thornei* and resistant to *P. neglectus*. The response of the Sarang variety, S-83-3 and SD-84-10 lines, was moderately resistant, and the Yavarous cultivar was susceptible to both species (Table 1).

Table 1. Reaction of wheat cultivars and lines against *Pratylencus thornei* and *P. neglectus* under greenhouse conditions

P. neglectus		Variety / Line	P. thornei		Variety / Line
Reaction*	RF		Reaction*	RF	
S	4.3	Trident	S	5.3	Warigal
S	2.2	Virinak	S	2.6	Chamran
S	1.3	Akbari	S	1.9	Virinak
S	1.1	Bam	S	1.6	2009-6-3
MR	0.8	D-84-5	S	1.5	S-87-21
MR	0.8	S-78-11	S	1.4	SS-85-10
MR	0.7	Aflak	S	1.3	Chamran 2
MR	0.6	VP5053	S	1.2	Khalil
MR	0.6	S-83-3	S	1.1	SD-84-12
MR	0.6	SD-84-12	S	1.1	Yavarus
MR	0.5	S-85-19	S	1	Sistan
MR	0.5	SD-84-10	S	1	S-85-19
MR	0.5	Kavir	MR	1	D-81-18
MR	0.5	Sarang	MR	1	Shabrang
MR	0.5	Barat	MR	1	D-84-9
R	0.5	S-92-19	MR	1	SS-85-18
R	0.4	Mehregan	MR	1	Sarang
R	0.4	Sistan	MR	1	S-83-3
R	0.4	Khalil	MR	0.7	SD-84-10
R	0.3	SD-84-8	MR	0.6	SS-85-14

R	0.3	D-83-1	MR	0.6	Mehregan
R	0.3	S-87-21	MR	0.6	D-87-9
R	0.2	SS-85-14	R	0.5	SS-85-11
R	0.2	S-87-22	R	0.5	S-87-20
R	0.2	Shush	R	0.5	D-83-1
R	0.2	SS-85-11	R	0.4	S-87-22
R	0.2	D-87-9	R	0.4	D-86-16
R	0.2	Chamran 2	R	0.4	D-84-5
R	0.2	SS-85-18	R	0.3	SS-85-6
R	0.1	S-80-18	R	0.3	Barat
R	0.1	Chamran	R	0.3	514
R	0.1	SS-85-20	R	0.3	GS50A
R	0.1	S-91-13	R	0.3	Yavarus
R	0.1	D-82-6	R	0.3	Akbari
R	0.1	514	R	0.3	S-84-14
R	0.1	D-83-8	R	0.3	S-91-13
R	0.1	Yavarus	R	0.3	Bam
R	0.09	D-87-15	R	0.2	D-87-3
R	0.09	S-87-20	R	0.2	D-85-17
R	0.09	SS-85-6	R	0.2	Kavir
R	0.07	SS-85-10	R	0.2	Shush
R	0.07	Shabrang	R	0.2	D-83-8
R	0.07	D-87-3	R	0.2	Behrang
R	0.06	Karkheh	R	0.1	Aflak
R	0.06	Behrang	R	0.1	S-92-19
R	0.05	Dena	R	0.1	D-87-15
R	0.05	D-81-18	R	0.1	SD-84-8
R	0.02	D-86-16	R	0.1	D-82-6
R	0.02	Star	R	0.1	Star
R	0.01	S-84-14	R	0.1	S-80-18
R	0.1	D-82-6	R	0.05	Karkheh
R	0	D-84-9	R	0.05	D-86-13
R	0	D-85-17	R	0	S-78-11
R	0	D-86-13	R	0	Dena
R	0	2009-6-3	R	0	SS-85-20

* S: Susceptible, R: Resistant, MR: Moderately Resistant. RF <1=R; 0.5<RF < 1 =MR; RF > 1 = S

Discussion

This study showed resistance reaction of 27 wheat cultivars and wheat lines to two species of root lesion nematodes, *P. thornei* and *P. neglectus* in greenhouse conditions. The wheat cultivar Dena had resistant reaction to both species confirming the results of previous study (Fayazi et al. 2012).

Similar results have been obtained in the response of several wheat cultivars and lines tested to *P. thornei* and *P. neglectus* species. In a study, the Akbari cultivar and S-80-18 line were resistant to *P. thornei* species, and Chamran cultivar was susceptible to *P. neglectus* species (Jahanshahi Afshar et al. 2010). In another research, in Khuzestan province, it was found that Star, Dena, and S-84-14, SS-85-20, and S-80-18 lines were resistant to both nematode species, which was consistent with the results of the present study (Fayazi et al. 2012). In the present study, there were differences in the response of 23 tested wheat cultivars and lines to *P. thornei* and *P. neglectus* species, which were similar to the research conducted in Morocco in the response of 25 wheat lines to *Hererodera avenae* and *P. penetrans* (Mokrini et al. 2015) and in Türkiye in the response of 74 cultivars of commercial wheat and wild Emmers to *P. thornei* and *P. neglectus* species (Toktay et al. 2015). The results of this research provide promising prospects for utilizing these sources of resistance in wheat improving program in future.

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Evaluation of resistance in Turkish wheat cultivars to spot blotch disease caused by *Bipolaris sorokiniana*

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Wheat is one of the most important food products required for human nutrition in the world. However, wheat production is adversely affected by many biotic and abiotic factors. Among the fungal diseases in wheat, *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*) is an economically important fungal pathogen causing spot blotch and common root rot on wheat plants, especially in winter wheat varieties. The pathogen affects seed, seedlings, roots, leaves, leaf sheath, and spike and causing serious losses in yield and quality of wheat under favorable conditions. The use of resistant varieties is considered the most beneficial and eco-friendly for managing spot blotch disease, although the pathogen can be controlled using several management strategies. This study evaluated host sensitivity of 55 wheat cultivars, widely grown in Türkiye against *B. sorokiniana*. Plants were spray inoculated with spore suspension of 1×10^4 conidia/mL until run-off. Disease severity of wheat plants was evaluated using a 1–9 scale 12 days after inoculation. The results revealed significant differences among the responses of wheat cultivars to the pathogen. The cultivars Anafarta and Koç-2015 were the most resistant with disease index of 1.63, while the cultivar Kırik was found to be the most susceptible cultivar with disease index of 7.97, followed by the cultivars Lancer and Doğu-88. Disease index of the other cultivars varied from 2.17 to 7.5. The results indicated that resistant wheat cultivars could serve as useful sources of genetic resistance in breeding studies for *Bipolaris sorokiniana*.

In vitro study of *Origanum majorana l.*, and *Salvia officinalis*: hydrodistillation, characterization and antifungal activity against *Fusarium culmorum*

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Summary

This work is part of the promotion of Moroccan medicinal plants known for their therapeutic virtues, it is devoted on the one hand to an ethnobotanical study and on the other hand to the extraction of essential oils, and the identification of the chemical composition whose objective is to study the antifungal activity of the oils. The chemical composition of essential oils was analyzed by GC-MS and major components for each oil were identified. For *Origanum majorana* , Terpinène-4-ol / 4-terpinéol (28,62 %), gamma-terpinène (12,87) et 4-carène (6,76). However for *Salvia officinalis*. alpha-thujone (31,38%) et L-Camphre (15,13%) are dominant. The antifungal activity of the two essential oils was carried out in vitro on *Fusarium culmorum* of wheat by the direct contact method. The comparison between the activities of the different EOs on *Fusarium culmorum* shows that the two plants show a great power to inhibit the mycelial growth of phytopathogenic fungi with an MIC of 4 µl/ml.

Introduction

In Morocco, wheat is the most consumed cereal, its consumption is estimated at 258 kg/year/person. Fungal diseases are one of the most important constraints for its production. Among them, fusariosis, which affects the yields but also the sanitary quality of the harvest by the presence of toxins in the grains. This disease causes damage and significant yield losses of up to 89% (Ma et al. ,2012).In order to reduce the impact of this disease, various control methods have been advocated, but biological control remains the most environmentally friendly. Thus, the essentials can act by inhibiting spore germination, mycelium elongation, sporulation and toxin production (Chami, 2005).

Methods

Plant material

Two different plants were used, they are *Origanum majorana* L., and *Salvia officinalis*. Indeed, O. *majorana* has an aromatic smell and a non-persistent taste (Muqaddas, 2016). Regarding S. *officinalis*, it is native to the eastern Mediterranean. It prefers warm and calcareous soils. It grows spontaneously or is cultivated throughout the Mediterranean basin from Spain to Türkiye, as well as in North Africa. These two plants are domesticated in the Guich tribal area of Rabat.

Fungal material

An isolate of *Fusarium culmorum* that has shown its virulence was chosen for the control tests. It was isolated from infected wheat crown and root samples.

Extraction of the essential oil

The extraction of the essential oil was carried out by following the technique of hydro distillation which is ensured thanks to an apparatus of the Clevenger type during 2 hours. Water and oil are separated by density difference. The essential oil obtained is kept at 4°C and in the dark. The yield of essential oil is determined in relation to the initial green matter.

Evaluation of the in vitro antifungal activity of essential oils

The antifungal activity of the essential oil of *O. compactum* and *Salvia officinalis* was evaluated according to the method reported by Adjou et al. 2012.

To the PDA medium, different initial concentrations (0.4, 0.6, 0.8, 1 µl/ml) and (0.8, 1, 1.5, 3 µl/ml) of essential oil of *O. compactum* and *S. officinalis* respectively were added with 2% Tween 80. Discs of 5mm diameter taken from the culture of a *Fusarium* isolate were deposited on the medium containing the EO. Controls containing only the PDA medium were also seeded in the same manner.

Three replicates were made of each concentration for the three isolates and then the set of replicate plates were incubated at 25°C and in the dark for 7 days.

Determination of MIC

The minimum inhibitory concentration (MIC) is relative to the concentration of the essential oil that inhibited the growth of the fungus. In order to determine the MIC of *O.majorana* and *S. officinalis,* PDA culture media at different concentrations were prepared and harvested in petri dishes. The dishes were submerged and incubated at 25°C for 7 days.

Determination of the PICD

The PICD is calculated according to the formula developed by Chang et al. in 1999

Gas chromatographic analysis

Gas chromatographic analyses were performed using a Perkin-Elmer autosystem GC apparatus, equipped with a splitter injector, two columns (50 x 0.22mm i.d. film thickness: 0.25 µm), apolar (BP-1, polymethylsiloxane) and polar (BP-20, polyethylene glycol) and two flame ionization detectors. The amount of sample injected was 0.5 µL from a solution containing 50 µL of mixture (essential oil or hydrolate extract or microwave extract or chromatography fraction) in 350 µL of CCl4

Results

Biological control with *O. majorana* essential oil

Antifungal activity

The evaluation of the percentage of inhibition for the *Fusarium culmorum* isolate is shown in Figure 1. It is noted that the degree of effectiveness of the essential oil of *O. majorana* on mycelial growth increases as the concentration of the essential oil increases (Fig. 1and Photo 1)

The study of the mycelial growth of the tested isolate of *Fusarium culmorum* in the presence of the essential oil of *Salvia officinalis*, shows a decrease of the mycelial growth synchronized with the increase of the oil concentration.(Fig.2 and Photo 2)

These results clearly show that the 2 parameters studied (minimum inhibitory concentration, percentage inhibition of diametral growth) are strongly influenced by the addition of an extract rich in active substances in the culture

Chemical composition of *Origanum majorana* L.

The chromatographic analysis of the essential oil of *Origanum majorana* revealed the dominance of Terpinene-4-ol or 4-terpineol (28.62%), gamma-terpinene (12.87) and 4-carene (6.76). The antifungal effect may be due to its predominant compounds (thymol and/or carvacrol) and dominant (terpene-4-ol and sabinere hydrate) (Busta et Jetter 2018).(Fig.3)

Chemical composition of *Salvia officinalis*

Gas chromatography coupled with mass spectroscopy (GC/MS) of the essential oil of *Salvia officinalis* has identified as major elements alpha-thujone (31.38%) and L-Camphor (15.13%). (Fig .4)

Other works have highlighted the inhibitory effect of an oxygenated monoterpene highly concentrated in the essential oil of sage (1,8- cineole) against pathogenic strains (Filipowic and al. 2003)

Discussion

Generally, the degree of antifungal activity is proportional to the concentration of the oil. Both essential oils of *O. majorana* and *S. officinalis* inhibited the mycelial growth of the tested *Fusarium* isolate. The results obtained confirm those presented by Grainge and Ahmed 1988, who showed that the Laminaceae possess antibacterial and antifungal activity thanks to the multitude of bioactive compounds they contain. The effectiveness of the essential oil of *O.majorana* may be due to its richness in terpinen-4-ol compound which is in accordance with the work of Vági et al. 2004. Similar studies (Monica et al. 2019) confirmed that terpene components are the agents with the highest antifungal potential in essential oils. Another study showed that the essential oil of *O. majorana* had very effective antifungal activity against the growth of *F. verticilliodies, F. graminearum, B. oryzae* and *C. lunata*.

The major component of *Salvia officinalis* is alpha-thujone (31.38%), this result is in agreement with Braga and al. 2001. Indeed, the same author found that the majority component of the essential oil extracted from stems, leaves and flowers is α-thujone. The other major components are α-thujone (23.11%), 1,8-cineole (16.19%), viridiflorol (10.48%) and thujone (6.73%).In contrast, some authors suggest that the different compounds of the essential oil can not act separately but the biological activity observed is the result of a probable synergy between the different constituents of the oil (Ben Khedher 2017).

In this study, the doses used were very effective in reducing the mycelial growth of the tested isolate. Normally, the diameters, speed and antifungal index of mycelial growth decrease each time the concentration of essential oil is increased until the total inhibition of the tested isolate, this is confirmed by the work of Gacem (2011) on methanoic and aqueous extract applied on *Aspergillus fumigatus*. The difficulty of developing an antifungal molecule is related on the one hand, to the ultrastructure of the antifungal cell which presents three barriers: the chitinous cell wall, the membrane ergosterols and the eukaryotic nucleus (Kolai et al.2012) and on the other hand, the antifungal molecules themselves which can generate resistance (Prasad and kapoor 2005).

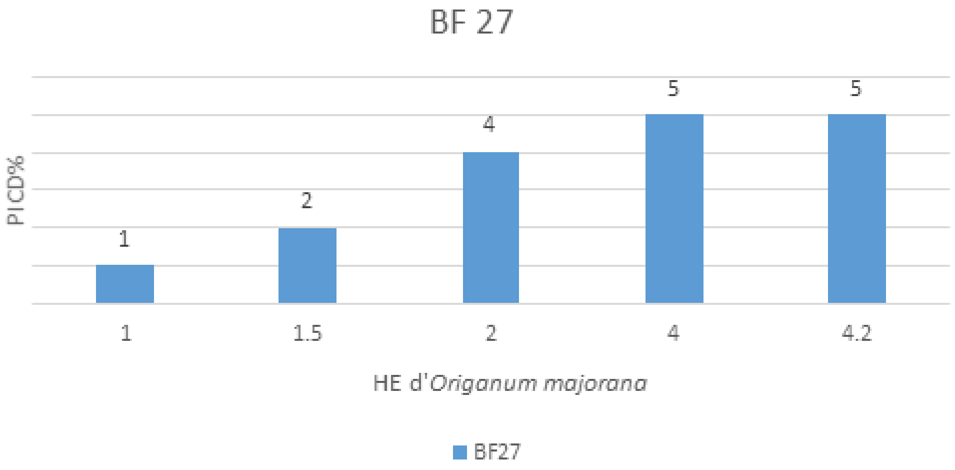

Figure 1. Minimum inhibitory concentration and percentage inhibition of diametral growth by *Origanum majorana*.

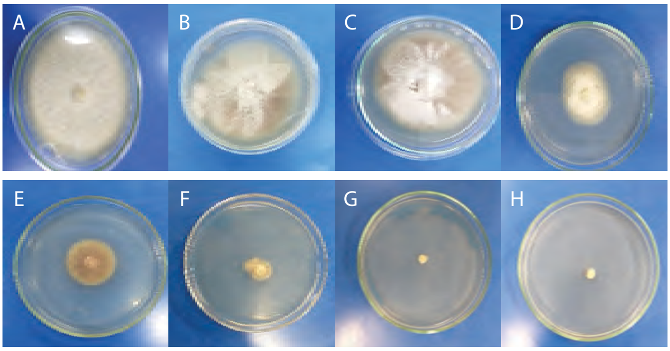

Photo 1. Effect of different concentrations of *O. Majorana* EO on the growth of BF27 after 7 days of incubation. A : 0µl/ml, B : 0.4 µ /ml, C :0.6 µ/ml, D:1µ /ml, E :1.1µ/ml, F :2µ/ml, G :4µ/ml, H : 4.2µl/ml.

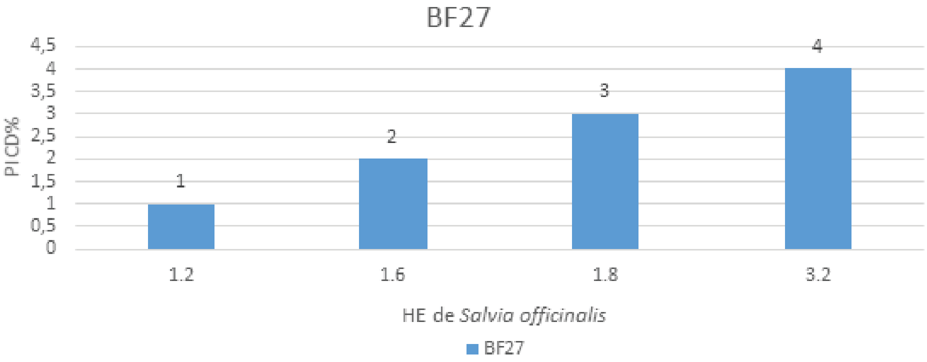

Figure 2. Minimum inhibitory concentration and percentage inhibition of radial growth by *Salvia officinalis.*

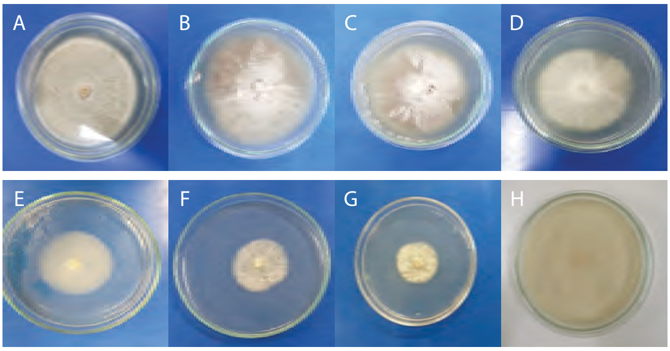

Photo 2. Effect of different concentrations of *S. officinalis* EO on the growth of BF27 after 7 days of incubation A : 0µl/ml, B : 0.4 µ /ml, C :0.6 µ/ml, D :1 µ/ml, E : 1.1µ /ml, F :1.8µ/ml, G:3,2µ/ml, H:4µl/ml.

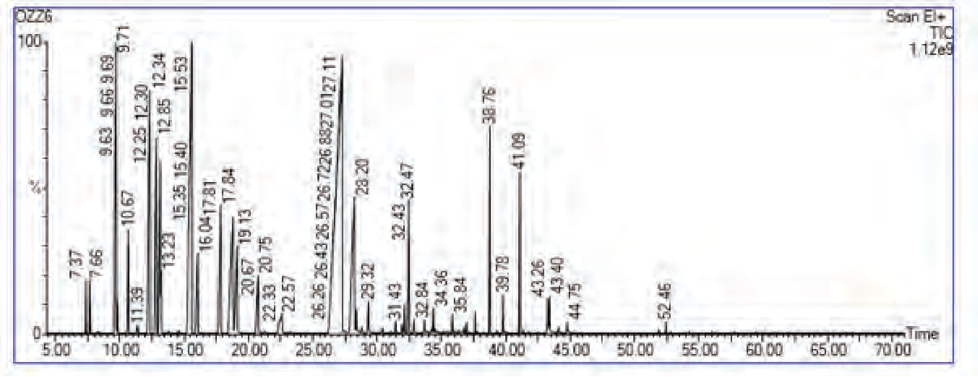

Figure 3. Chromatogram of the essential oil of *Origanum majorana.*

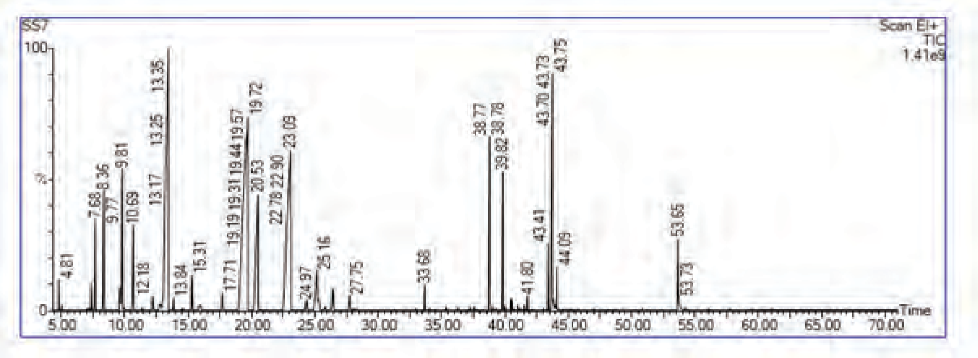

Figure 4. Chromatogram of the essential oil of *Salvia officinalis.*

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Bipolaris sorokiniana associated with common root rot in Azerbaijan and Kazakhstan

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Wheat (*Triticum* spp.) is the third most important crop in terms of global production, with an average annual production of almost 219 million ha and yielding 760.9 million tons, after maize and rice. However, a complex of fungi attacking the crown and root tissues of wheat causes a serious problem and significant yield reductions in wheat. Although most prevailing species in this complex can change yearly and regionally in dryland winter wheat production areas, *Bipolaris sorokiniana* Shoemaker (teleomorph: *Cochliobolus sativus*) is one of the dominant species and causes spot blotch and common root rot (CRR) on wheat plants, especially in winter wheat varieties. Comprehensive surveys were conducted to identify *B. sorokiniana* associated with CRR of wheat throughout the main wheat-growing areas of Azerbaijan and Kazakhstan in 2017 and 2019, respectively. As a result of the classification of species based on morphological and molecular tools, 96 and 547 isolates were identified as *B. sorokiniana* for Azerbaijan and Kazakhstan, respectively. All surveyed regions for these countries were contaminated with this pathogen. Some representative isolates of *B. sorokiniana* were tested for their ability to cause disease and produced moderate disease severities on cultivar Seri 82 (*Triticum aestivum*, bread wheat). The percent incidence of *B. sorokiniana* isolates in all isolates was 15.2% for Azerbaijan, whereas, for Kazakhstan, *B. sorokiniana* was the most frequently recovered species in the three regions surveyed, with an isolated frequency of 44.80%. The results of the current study provide crucial and helpful information to improve disease management strategies against CRR of wheat in these countries.

Enhanced damage severity with simultaneous inoculation of root lesion nematodes, *Pratylenchus* spp. and root and crown rot fungi, *Fusarium* spp. on wheat in greenhouse experiments

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Summary

Wheat is an important source of energy and constitutes the main food of about 40% of world's population. Soil borne diseases, especially caused by root lesion nematodes, RLNs, (*Pratylenchus* spp.) and Fusarium root and crown rot, FRCR, are the most important causal agents in wheat yield reduction worldwide and likewise in some parts of Iran. The interaction of two prevalent RLN species *P. neglectus* and *P. thornei*, in combination or individually with *F. culmorum* and *F. pseudograminearum*, the two main agents of FRCR of wheat, were evaluated on wheat in a completely randomized design with six treatments (totally 24 treatments for four experiments) which were replicated four times *in vivo*. Results showed that all of the studied pathogens caused significant reduction of measured parameters. Increase of damage on wheat plants when both the fungus and nematode were combined together, compared to either pathogen individually, indicated a positive interaction among the nematodes and fungi. In all experiments, maximum reductions of plant growth parameters were recorded in sequentially inoculated plants where nematodes were inoculated one week before the fungi. *P. thornei* caused more damage than *P. neglectus* and its interaction with *F. pseudograminearum* caused maximum reduction in plant growth indices.

Introduction

Wheat is an important source of energy and constitutes the main food of about 40% of world's population. Plant parasitic nematodes, specially root lesion nematodes, RLNs (*Pratylenchus* spp.), are one of the most important factors in wheat yield reduction. These nematodes are capable of causing direct damage through feeding on the host plant and indirect damage by interacting with soil born microorganisms specially root-rot fungi. *Pratylenchus neglectus* and *P. thornei* are RLNS that have a worldwide distribution. *P. thornei* causes 32 to 70% damage to the wheat crop annually in the world. Root and crown rots of wheat which are caused by species of *Fusarium, Pythium, Rhizoctonia, Drechslera, Bipolaris, Gaeumannomyces* and *Oculimacula* are other limiting factors in wheat production which causes at least 13% yield losses. *Fusarium* species are the main agents of wheat root and crown rot in Iran. Kazemi (2002) investigated *Fusarium* species associated with root and crown of wheat in Tehran province of Iran. The result of pathogenicity tests showed that *F. culmorum* and *F. pseudograminearum* were pathogenic in Falat cultivar. Nicol *et al*. (2004) reported simultaneous infection of wheat crop to root lesion nematode and root rot fungi in Hamedan, Kermanshah, East Azarbayejan, Khorasan and Golestan provinces of Iran. Tanha Maafi *et al*. (2009) studied cereal cyst nematodes (CCNs), root rot pathogens (RRPs) and RLNs affecting cereal production in Iran. Their results showed that RRP including

F. pseudograminearum, *F. culmorum*, *Bipolaris sorokiniana* and *Rhizoctonia cerealis* were frequently found in combination with the RLN and/or CCN. The objective of present study was to accurately determine the interaction between root lesion nematodes (*P. neglectus* and *P. thornei*) and root and crown rot fungi (*F. pseudograminearum* and *F. culmorum*) on wheat under *in vivo* condition.

Methods

Materials and methods were briefly as follows: 1. Inoculum preparation: Inocula of FRCR, *F. pseudograminearum* and *F. culmorum*, were increased on wheat grains. Propagation of population of RLNs, *P. neglectus* and *P. thornei*, was carried out in carrot culture medium. 2. Planting: Susceptible Sardari wheat seeds, after surface disinfestation and germination, were planted in pots, as following treatments. 3. Treatments: For each species of fungus and/or nematode, a total of four experiments were conducted based on completely randomized design with six treatments (a total of 24 treatments for four experiments) and four replications, via pot soil infection, under controlled condition. Treatments were included with: a) only fungus inoculation. b) only nematode inoculation. c) fungus+nematode inoculation, simultaneously. d) first nematode and then, after one week, fungus inoculation. e) first fungus and then, after one week, nematode inoculation and f) control (without fungus and nematode inoculation). 4. Assessment: The plant growth parameters including lengths of stem and root, weights of stem and root and numbers of tillers were calculated for each experiment. Also the pathologic parameters including numbers of nematodes in soil and plant and root rot index (Wallwork *et al*. 2004) were evaluated for all experiments. The data obtained from all parameters were statistically analyzed by using SPSS 22 software. Mean of treatments were compared with LSD at 5% significance levels.

Results

RLN, *P. thornei*, was able to reduce significantly the measured plant growth parameters, while the FRCR, *F. culmorum*, could only significantly reduce the length of stem and number of tillers. Significant reductions of growth parameters in the presence of both agents were much greater than when plants were inoculated with each one alone. The maximum reductions were observed when nematode inoculation was done one week before the fungus (Table 1). In inoculated treatments by both agents, nematodes increased the severity of fungal root rot and its severity was higher in nematode treatment one week before the fungus. *P. thornei* and *F. pseudograminearum*, each alone caused a significant reduction in growth parameters, which they were greater in nematode-inoculated plants. As in the first experiment, maximum reductions in all parameters were observed with sequential nematode inoculation one week before the fungus (Table 2).

Significant reduction was not observed in the length of stem and root of plants inoculated by any of the agents, *P. neglectus* and/or *F. culmorum*, although the presence of both agents caused a significant reduction in all measured parameters. Like the previous experiments, maximum reductions were observed in sequential inoculationwith nematode one week before the fungus (Table 3). RLN, *P. neglectus*, and FRCR, *F. pseudograminearum*, each alone caused significant reductions in growth parameters. These reductions were greater in the presence of two agents. In this experiment as well, maximum reductions in all parameters were observed with sequential nematode inoculation one week before the fungus (Table 4).

Table 1. Interaction effect of *Pratylenchus thornei* and *Fusarium culmorum* on wheat under controlled conditions

Treatment[1]	Parameters[2]							
	Length (cm)		Weight (gr)		Number of			Root rot index
	Stem	Root	Stem	Root	Tiller	Nematode/ 1kg soil	Nematode/ whole root	
C	60.75a	34.25a	76.50a	42.50a	10.25a	-	-	-
FC	55.75b (-8.23)	31. 75a (-7.30)	72.50a (-5.23)	40.50a (-4.70)	9.00b (-12.19)	-	-	3.00c
PT	49.50c (-18.51)	26.50b (-22.62)	61.75b (-19.28)	32.00b (-24.70)	7.50bc (-26.83)	1500a	1610a	2.75c (-8.33)
PT+FC	46.00cd (-24.28)	26.50b (-22.62)	51.25c 33.00)(-	28.50c (-32.94)	6.00c (-41.46)	1275b (-15.00)	1467b (-8.88)	4. 00b (+33.33)
PT⇨FC	26.75e (-55.97)	16. 25c (-52.55)	27.00d (-64.70)	16.75d (-60.56)	4.50d (-56.10)	1500a (00.00)	1125b (-30.12)	4.75a (+58.33)
FC⇨PT	42.50d (-30.04)	25.50b (-25.55)	46. 25c (-39.54)	27.75c (-34.70)	6.75c (-34.15)	1100c (-26.67)	1180b (-26.70)	4.00b (+33.33)

[1] C= Control, PT= *Pratylenchus thornei*, FC= *Fusarium culmorum*, PT+FC=PT & FC inoculated simultaneously, PT⇨FC= PT one week before FC, FC⇨PT= FC one week before PT.

[2] Means with the same letters in each column are not significantly different at 5% level according to LSD test. Values in brackets are percent change in comparison to control.

Table 2. Interaction effect of *Pratylenchus thornei* and *Fusarium pseudograminearum* on wheat under controlled conditions

Treatment[1]	Parameters[2]							
	Length (cm)		Weight (gr)		Number of			Root rot index
	Stem	Root	Stem	Root	Tiller	Nematode/ 1kg soil	Nematode/ whole root	
C	61.00a	33.50a	76.25a	43.00a	9.75a	-	-	-
FP	54.25b (-11.06)	27.75b (-17.16)	68.50b (-10.16)	37.75b (12.20)	8.25b (-15.38)	-	-	2.00c
PT	44.00c (-27.86)	25.50bc (-23.88)	54.75c (-28.19)	32.50c (-24.41)	6.50c (-33.33)	1475a	1622a	2.00c (00.00)
PT+FP	40.00cd (-34.42)	22.25c (-33.58)	42.50d (-44.26)	27.50d (-30.04)	7.00bc (-28.20)	1200b (-18.64)	1302b (-19.72)	3.25b (+62.50)
PT⇨FP	28.00e (-54.10)	15.00d (-55.22)	22.75e (-70.16)	15.50e (-63.95)	3.50d (-64.10)	1500a (+1.69)	1025c (-36.80)	4.50a (+125.0)
FP⇨PT	36.75d (-39.75)	26.50bc (-20.89)	40.50d (-46.88)	27.50d (-36,04)	6.25c (-35.90)	1150b (-22.03)	875c (-46.05)	3.00b (+50.00)

[1] C= Control, PT= *Pratylenchus thornei*, FP= *Fusarium pseudograminearum*, PT+FP=PT & FP inoculated simultaneously, PT⇨FP= PT one week before FP, FP⇨PT= FP one week before PT.

[2] Means with the same letters in each column are not significantly different at 5% level according to LSD test. Values in brackets are percent change in comparison to control.

Table 3. Interaction effect of *Pratylenchus neglectus* and *Fusarium culmorum* on wheat under controlled conditions

Treatment[1]	Parameters[2]							
	Length (cm)		Weight (gr)		Number of			Root rot index
	Stem	Root	Stem	Root	Tiller	Nematode/ 1kg soil	Nematode/ whole root	
C	60.75a	31.75a	76.75a	43.00a	10.75a	-	-	-
FC	57.25a (-5.76)	29.50a (-7.08)	74.00ab (-3.58)	30.50bc (-29.06)	9.50b (-11.62)	-	-	2.25c
PN	55.00a (-9.46)	28.50a (-10.23)	70.25b (-8.46)	38.25b (-11.04)	8.75abc (-18.60)	1300a	1435a	2.25c (00.00)
PN+FC	48.25b (-20.75)	24.50b (-22.83)	64.25c (-16.28)	33.50bc (-22.09)	7.25c (-32.55)	1125b (-13.46)	1162ab (-19.02)	3.00b (+33.33)
PN⇨FC	33.50d (-44.85)	21.25c (-33.07)	36.75d (-52.11)	25.50d (-47.67)	4.50d (-58.13)	850c (-34.61)	1515a (+5.57)	4.75a (+111.11)
FC⇨PN	42.75c (-29.62)	24.50b (-22.83)	64.00c (-16.61)	32.00c (-25.58)	7.75bc (-27.90)	950bc (-26.92)	997b (-30.52)	3.00b (+33.33)

[1] C= Control, PN= *Pratylenchus neglectus*, FC= *Fusarium culmoum*, PN+FC=PN & FC inoculated simultaneously, PN⇨FC= PN one week before FC, FC⇨PN= FC one week before PN.

[2] Means with the same letters in each column are not significantly different at 5% level according to LSD test. Values in brackets are percent change in comparison to control.

Table 4. Interaction effect of *Pratylenchus neglectus* and *Fusarium pseudograminearum* on wheat under controlled conditions

Treatment[1]	Parameters[2]							
	Length (cm)		Weight (gr)		Number of			Root rot index
	Stem	Root	Stem	Root	Tiller	Nematode/ 1kg soil	Nematode/ whole root	
C	59.50a	33.00a	76.50a	43.75a	10.75a	-	-	-
FP	53.25b (-10.50)	27.50b (-16.66)	64.50b (-15/68)	37.50b (-14.28)	8.25b (-23.25)	-	-	1.00c
PN	55.75ab (-6.30)	25.00b (-24.24)	62.50b (-18.30)	36.00bc (-17.71)	8.00b (-25.58)	1250a	1532a	1.00c (00.00)
PN+FP	46.50c (-21.84)	22.25c (-32.57)	51.00c (-33.33)	32.25cd (-26.28)	7.00b (-34.88)	1025b (-18.00)	1447a (-5.55)	2.25b (+125.00)
PN⇨FP	30.25e (-49.15)	19.50d (-40.90)	33.75d (-55.88)	28.00e (-36.00)	4.25c (-60.46)	700c (-44.00)	855c (-44.20)	3.25a (+225.00)
FP⇨PN	40.50d (-31.93)	22.25c (-32.57)	60.75b (-20.58)	30.00de (-31.42)	7.75b (-27.90)	800c (-36.00)	975b (-36.35)	2.00b (+100.00)

[1] C= Control, PN= *Pratylenchus neglectus*, FP= *Fusarium pseudograminearum*, PN+FP=PN & FP inoculated simultaneously, PN⇨FP= PN one week before FP, FP⇨PN= FP one week before PN.

[2] Means with the same letters in each column are not significantly different at 5% level according to LSD test. Values in brackets are percent change in comparison to control.

Discussion

RLNs are internal parasites, polyphagous and migratory. FRCR, specially *F. culmorum* and *F. pseudograminearum*, are the main agents of wheat root and crown rot in Iran. It was found that inoculation with both pathogens, RLNs and FRCR, increased the damage severity to wheat in all experiments. The disease severity was observed to be higher in treatments where both fungus and nematode were combined together. Increased symptoms of root rot indicated synergistic interaction between nematodes and fungi under studied, in their simultaneous and sequential inoculation. Therefore, a positive interaction between nematodes and fungi was proved and this finding was consistent with the report of Taheri *et al*. (1994). The greatest damage to the plant was observed with sequential inoculation of agents, nematode one week before the fungus, which can be attributed to biochemical and physiological changes caused by nematode activity and providing more nutrition suitable for fungal activity. Indeed, RLNs aggravated root rot and these symptoms were more severe in sequential inoculation (nematode one week before the fungus). Decreased nematode population in sequential inoculation of agents, fungus one week before nematode, can be due to secretion of anti-nematode chemicals by fungus and/or infected plant (Gommers 1981). The results of our study are consistent with the findings of other researchers (Chandel and Sharma 1989, Bhattarai *et al*. 2009, Hajihassani *et al*. 2013).

In conclusion, it was shown that both FRCR and RLNs cause significant damage or yield loss in wheat fields, so it is important to consider both pathogens when designing integrated disease management (IDM). The crop rotation with non-host crops and use of resistant wheat varieties in field, can be very important and key methods in IDM program at areas where root rot diseases complex are present and cause yield losses.

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Investigating the suppressive effects of entomopathogenic nematodes on root knot nematodes attacking wheat

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Summary

This study aimed to investigate the suppressive effects of entomopathogenic nematodes on root knot nematodes attacking wheat. The root knot nematode (RKN) was isolated from infested roots of cucumber grown in uplands area in Jordan. The isolate was identified as *Meloidogyne javanica* based on morphological characters. A previously identified Jordanian isolate of the entomopathogenic nematodes (EPN) *Steinernema feltiae* was used in this study. A laboratory experiment was performed to study the effect of the EPN isolate on penetration of second stage juveniles (J2s) of RKN *M. javanica* into the roots of wheat cultivar Acsad 65 grown on water agar medium. The observation revealed that the studied EPN isolate reduced the penetration of the J2s of RKN *M. javanica* into roots of wheat plant. No or very few galls on wheat roots were found whether IJs of EPN were added preceding or at the same time of RKN inoculation.

Growth chamber experiments were conducted to investigate the suppressive effects of EPN on RKN on two wheat cultivars, Horani Nawawi and Acsad 65. Results showed that the application of the isolate of EPN, *S. feltiae,* alone or in combination with RKN increased both foliage and root weight over those of RKN only treatment. The application of EPN *S. feltiae*, suppressed the galling and eggmass production on wheat roots compared to plants inoculated with RKN alone.

Introduction

Entomopathogenic nematodes, belonging to the genera *Steinernema* and *Heterorhabditis* are soil organisms adapted to most climatic conditions in hot, temperate, and cold zones (Bhat et al. 2020, Steiner 1996, Stock et al. 1999). In nature, EPN are obligate parasites that attack wide range of soil insects (Kaya and Gaugler 1993). Currently several strains of EPN species are being used commercially to control insect pests because they are effective and environmentally safe to be used.

In addition to its insecticidal effect, EPN are recorded to suppress plant parasitic nematodes including root knot nematodes (El Aimani et al. 2022, Bader 2013, Grewal et al. 1999, Perry et al. 1998, Smitley et al. 1992, Tsai and Yeh 1995). It is of great importance to use safely and friendly control means such as these EPN to suppress several pests (insects and nematodes). The EPN *S. feltiae* was recorded to control wheat pests such as wheat stem sawfly, *Cephus cinctusow* and fruit fly, *Oscinella frit* (Portman et al. 2016, Imperiali et al. 2017). The aim of this study was to investigate the suppressive effects of a Jordanian isolate of the entomopathogenic nematode *S. feltiae* on root knot nematodes, *M. javanica* attacking wheat.

Methods

A Jordanian isolate of the EPN species, *S. feltiae*, was used in this study. This isolate was originally recovered from oak plantings in Malka, Irbid area (Stock et al. 2008). This isolate was reared on wax moths to maintain the EPN culture. The IJs of the studied EPN was recovered from infected insects by using white trap and the recovered IJs were collected and stored at 10-20 ºC (Orozco et al. 2014). Fresh IJs was used in this study. While the root knot nematode (RKN) was isolated from infested roots of cucumber grown in uplands area in Jordan. The isolate was identified as *M. javanica* based on morphological characters (Jepson 1987). Hatched second stage juveniles (J2s) and handpicked eggmasses were used as the nematode inocula in the different assays.

In vitro experiment was conducted to investigate effect of the entomopathogenic nematode, *S. feltiae*, on penetration of J2s of the root knot nematode, *M. javanica*, on wheat roots grown on water agar. Seeds of the wheat cultivar Acsad 65 were surface sterilized using 1.5% sodium hypochlorite, and were seeded on 2.3% water agar. After three days of incubation, some root tips were either inoculated with only RKN *M. javanica* (100J2s, or three eggmasses), 100 IJs of the EPN *S. feltiae* and three eggmasses of *M. javanica* simultaneously, or 100 IJs of *S. feltiae* precede (3 days) 100 J2s of *M. javanica*. Each treatment was replicated five times. Each replicate was completely randomized in an incubator at 24 ºC. Roots were observed hourly for 8 hours, then every 24 hours up to one week when root galls were obvious.

Growth chamber experiments were carried out to study the effect of time of EPN application on galling of wheat roots and on reproduction of RKN. Two wheat cultivars, Horani Nawawi and Acsad 65 were used in this study. One inoculum level, 200 IJs of EPN were added to the 12 cm open tube and five eggmasses/pot of the RKN *M. javanica* was used. Both inocula, EPN and RKN, were applied as soil drenching to 12 cm open tubes filled with soil and planted with wheat seedlings. The EPN was applied either preceding RKN inoculation by one week or simultaneously with the RKN inoculation. Wheat plants alone or inoculated only with RKN served as check controls. Each treatment was replicated five times. Wheat tubes were maintained under optimum conditions and were checked periodically to monitor the growth of the wheat. Plants were harvested four weeks after RKN inoculation and plant parameters and root galling were scored.

Results and discussions

Our observation revealed that whenever IJs of EPN*, S. feltiae* stayed around the roots and roots tips, the J2s of the root knot nematode, *M. javanica*, were away from root tips and did not enter the roots in most cases and thus no galls were formed. Similar results were noticed by other researchers and they speculated that both EPN and its symbiotic bacteria repelled the J2s of RKN by producing toxins (Bader 2013, Bird and Bird 1986, Grewal et al. 1999, Perez and Lewis 2002). When EPN were absent, J2s of RKN reached root tips and penetrated the roots and eventually the root galls were formed in wheat cultivar. However, no or very few galls were found on all other treatments whether IJs of EPN were added preceding or at the same time of RKN inoculation.

The growth chamber experiments showed that application of the 200 IJS of the Jordanian isolate of the EPN *S. feltiae* increased foliage and root growth of the two wheat cultivars even when plants were inoculated with RKN. Similarly, Bader (2013) and Hussaini et al. (2009) also reported that strains of EPN increased the root, shoot and yield weights of cucumber and tomato plants while, RKN decreased growth and yield of plants. This enhancement of plant parameters might be explained by EPN species that are associated with symbiont bacteria that may colonize plant roots and enhance plant growth.

Results also showed that the application of the 200 IJS of the Jordanian isolate of EPN *S. feltiae* inhibited or reduced the galling of wheat roots. This inhibition or reduction varied with wheat cultivar and time of application whether IJs of EPN were added preceding or at the same time of RKN inoculation. Suppression of RKN effects on different crops by the use of EPN was reported from growth chambers, glasshouse, and field studies (Bader 2013, El Aimani et al. 2022, Grewal et al. 1997, Grewal et al. 1999, Hussaini et al. 2009, Ishibashi and Choi 1991, Lewis et al. 2001,). Fallon et al. (2002) conducted a greenhouse experiment and showed that the penetration of *M. javanica* was suppressed in soybean by *S. feltiae*. Lewis et al. (2001) demonstrated that *S. feltiae* reduced the number of galls in roots of tomato, egg production per plant, and egg hatch of *M. incognita*. Bader (2013) showed that both *S. feltiae* and *H. bacteriophora* suppressed penetration and egg production of both *M. javanica* and *M. incognita* in addition to the inhibition or reduction of root galling in cucumber.

In conclusion, field experiments will be conducted to confirm laboratory and growth chamber findings. Further studies should be implemented on other wheat pests including cereal cyst nematode, root lesion nematodes, and insect pests to augment the efficacy of this Jordanian isolates of EPN and to be included as a component in wheat crop management.

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Molecular initiation of wheat resistance to soil-borne pathogens

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Summary

Chitin is a major component of fungal cell walls and the eggshell of the nematodes. It serves as a microbe-associated molecular pattern (MAMP) for the detection of various potential pathogens in the innate immune systems of plants. Chitin elicitor-binding protein (CEBiP), plasma membrane glycoprotein with LysM motifs that functions as a cell surface receptor for chitin elicitor, is associated with a receptor-like kinase, *CERK1*, which is required for signaling through the plasma membrane into the cytoplasm. *CERK1* is a plasma membrane protein containing three LysM motifs in the extracellular domain and an intracellular Ser/Thr kinase domain with autophosphorylation/myelin basic protein kinase activity. The Cereal cyst nematode *Heterodera filipjevi* and the crown rot caused by *Fusarium* spp. are important diseases facing cereal production around the world. They attack cereal crops and cause significant yield loss. Yield losses accelerate when those diseases coexist with other abiotic factors such as drought. Under stress conditions, plants adopt certain genetic defense mechanisms. Considering the importance of the early response genes' role in plant resistance to stress, here the expression pattern of the *CERK 1* gene was studied in the root of two wheat cultivars under drought, *Fusarium culmorum,* and *Heterodera filipjevi,* across 3-time points including 24 h, 48 h, and 7days after stresses. The highest expression was observed at 24 h and 7d after stresses, respectively. *CERK1* gene was significantly up-regulated under most treatments in both varieties. The highest gene expression happened under nematode/fungus treatment in resistant variety to *F. culmorum*. Also, the highest gene expression of *CERK1* in resistant variety to *H. filipjevi* (Silverstar), was observed under nematode/fungus treatment 24h after inoculation. *CERK1* expression showed reduction under the combination of drought/pathogen stresses, compared with *CERK1* expression by each single pathogen stress in both varieties, this result confirms the antagonistic effect of abscisic acid (ABA) signaling pathway on salicylic acid (SA) and jasmonic acid (JA)-ethylene signaling pathways.

Introduction

Plants trigger various defense reactions against invading pathogens upon perception of so-called microbe-associated molecular patterns [MAMPs; also known as pathogen associated molecular patterns (PAMPs)] (Miya et al. 2007). Chitin is a main component of the cell wall of fungal pathogens, the exoskeletal cuticle and gut lining of insects and the egg shell of the nematode. Previous studies indicated that CEBiP (chitin elicitor-binding protein) and its partner LysM-RLK (receptor- like kinase) chitin elicitor receptor kinase1 (CERK1) are involved in plant immunity by recognizing the chitin oligosaccharides of the cell wall of fungal pathogens through extracellular LysM domain. It has been shown that the receptor-like kinase (RLK) pattern recognition receptor (PRR) protein CERK1 perceives the fungal pathogen activated molecular patterns (PAMPs) chitin to trigger a PAMP-triggered immunity (PTI) response. The PTI signaling requires its dimerization with the receptor-like protein CEBiP1 through direct binding with chitin (Kong et al. 2015).

Wheat as a good source of carbohydrate, protein, sugar, fat, fibre and minerals, provides half of the energy requirements of the human population (Zhang et al. 2017). Drought is one of the major causes of dramatic yield loss in wheat fields (Melloul et al. 2014). *Heterodera filipjevi*, a species of cyst nematode that is parasitic to cereals, is now widely recognized as a major nematode pest of wheat in cereal production areas especially in temperate climates and semi-arid areas in Europe and West Asia (Hajihassani et al. 2013). *Fusarium culmorum* is a ubiquitous soil-borne fungus able to cause foot and root rot and Fusarium head blight on different small-grain cereals, in particular wheat and barley. It causes significant yield and quality losses (Scherm et al. 2013).

The present study was conducted for comparing the expression pattern of *CERK1* gene in roots of two bread wheat under drought, *H. filipjevi* and *F. culmorum* stresses in single and interaction status of stresses.

Methods

Plant materials, growth and stress conditions: Two wheat accessions were studied for gene expression analysis. These accessions were 2–49 (resistant to *Fusarium*) and 'Silverstar' (resistant to *Heterodera*). Through primary studies by the Soil Borne Pathogens program at the CIMMYT-Türkiye office, a set of wheat germplasm with differing levels of resistance/ tolerance to CCN, crown rot, and drought was treated by *H. filipjevi, F. culmorum*, and drought, independently and simultaneously (Ahmadi et al. 2022).

To test the level and pattern of gene expression under simultaneous biotic and abiotic stresses and compare the plant's response to independent stress conditions, these two accessions were treated on the seven-day-old seedling. Drought stress applied in this study included 40% of field capacity (Ahmadi et al. 2022). Cereal cyst nematode, *H. filipjevi,* sample collected by CIMMYT-Türkiye (Dababat *et al.* 2014) was used in this study, and each seedling was inoculated with 400 freshly hatched J2 of *H. filipjevi* (Ahmadi et al. 2022). An identified isolate of *F. culmorum,* causing crown rot, from CIMMYT-SBP collection was used as the third stress factor (Ahmadi et al. 2022). Each seedling was inoculated with *F. culmorum* spores suspended in water (1 × 106 spores mL^{-1}) (Erginbas-Orakci et al. 2016).

Experimental design and sampling: Sampling of wheat root tissue, for RNA extraction and gene expression analysis, was performed three times after exerting stress conditions. These three sampling times are important stages in the early transcriptional response of plants to stress (Kong et al. 2015). Sampling subtended six replicates (two biological and three technical). Each biological replicate sample contained five seedlings.

We ran a factorial analysis of variance (ANOVA) in this study. The experimental design consists of five factors including genotype (2–49 and Silverstar), two drought levels (40% stress and non-stress), two CCN levels (inoculated and non-inoculated), two crown rot levels (inoculated and non-inoculated), and sampling time (24h, 48h, and one week after treatment). Mean comparison was performed using the least significant difference test (LSD) ($P \leq 0.05$).

RNA isolation, cDNA synthesis and qRTPCR: Root of plants were sampled at 24 h, 48h and 8 day post treatments. Total RNA was extracted from samples using the TRIzol Reagent (Dena Zist Asia), and were treated with DNase I. Total RNA samples were used to synthesize first strand cDNA by manufacturer's instructions (cDNA synthesis, Takara kit).

Real-time PCR was performed on QIAGEN's real-time PCR cycler, the Rotor-Gene Q. The forward and reverse primers for RT-PCR were designed from conserved domain of *Triticum aestivum* and *Oryza sativa* in NCBI database.

Results

The results revealed that fungus and nematode treatments caused increase in *CERK1* expression The results of mean comparison showed *CERK1* expression in Silverstar variety was higher than gene expression in '2-49' variety. The highest gene expression in roots of variety '2-49' was observed under nematode-fungus combination 24h post treatment and in second rate under single fungus treatment while the lowest gene expression was under drought-nematode combination and under single drought stress.

The highest *CERK1* expression in Silverstar variety was observed under combination of fungus and nematode in 24h post treatment while the lowest gene expression was under single drought stress and single fungus stress.

Up-regulation of *CERK1* in resistant variety to *F. culmorum*, '2-49' was higher than up-regulation in Silverstar when were exposed to fungus stress and its interaction with other stresses. Also gene expression in resistant variety to *H. filipjevi*, Silverstar was higher than gene expression in '2-49' variety under nematode stress and it's interaction with other stresses.

Discussion

During evolutionary processes, plants have developed sophisticated mechanisms of defense against pathogens. In pattern-triggered immunity (PTI), conserved virulence factors such as chitin, the main building block that makes up the cell wall in pathogenic fungi, are recognized by the host plant. The wheat genome contains one gene encoding CEBiP and two genes encoding *CERK1*. Chitin elicitor-binding protein is a membrane protein that has been first identified in rice. The lysine motif in the extracellular domain of CEBiP recognizes and binds chitin, but CEBiP does not have an intracellular signaling domain, and it is unable to initiate an immune signal. The CERK1 transmembrane protein comprises an extracellular domain with a lysine motif and an intracellular kinase domain, and it is capable of inducing a signal transduction cascade (Duba et al. 2018). *CERK1* played potential roles in defense responses to CCN (Kong et al. 2014) and *Fusarium*. Spp. (Bertini et al. 2003 and Desmond et al. 2006).

The results of present study showed that *CERK1* gene has an effective role in early defence response to soil borne pathogens. The highest gene expression was observed 24h after stresses. One of the typical time points in cereal cyst nematode virulence is penetration stage (24h), in this stage parasitic juvenile 2 (J2) penetrates to root and migrates through cortical cells in 72h (3d) (Kong et al. 2015). Fungal hyphae had extensively colonized both the rhizodermal cell layer 24h post infection and the first layer of cortex cells by inter- and intra-cellular growth. Colonization of the cortex continued and by 48 h post infection a dense mycelial network was present inside the lumen of cortical cells in the region adjacent to the inoculation point. However, the fungus did not penetrate the endodermis, the last layer of cortical cells, and was absent from the protoxylem and metaxylem vessels in the stele. (Beccari et al. 2011). Kong et al. (2015) reported that the *CERK1* gene under infection of wheat roots with *H. avenae* was up-regulated, with the highest up-regulation in 24h after inoculation. They reported that *CERK1* played potential roles in defense responses to CCN. Shimizu et al. (2010) reported that OsCERK1 is a key component for chitin elicitor signaling in rice. The OsCERK1 knockdown cell lines mostly lost the ability to generate ROS, to induce the expression of defense genes and to synthesize phytoalexins in response to chitin oligosaccharide elicitor.

In this present study, combination of drought/pathogen caused reduction in CERK1 expression compared with expression under each single pathogen stress, indicating the antagonistic effect of abscisic acid (ABA) signaling pathway on salicylic acid (SA) and jasmonic acid (JA)-ethylene signaling pathways.

ABA is an important plant hormone that plays signaling roles in different processes of plant growth and development as well as in plant responses to various abiotic stresses like drought stress. Plant resistance to biotrophic pathogens such as cyst nematodes is classically thought to be mediated by the salicylic acid (SA) pathway, and by contrast, resistance to necrotrophic pathogens such as *Fusarium* spp. is controlled by jasmonic acid (JA) and ethylene (ET) pathways. Abscisic acid (ABA) signaling pathway inhibits salicylic acid (SA) and jasmonic acid (JA)-ethylene signaling pathways (Anderson et al. 2004).

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Up-regulation of *WRKY29* gene under simultaneous biotic and abiotic stresses in the root of wheat

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Summary

Wheat (*Triticum aestivum* L.) is the most widely grown crop in the world. It grows under different climatic and agronomic conditions and faces various biotic and abiotic stresses. To cope and survive under these conditions, wheat uses a variety of defense and tolerance mechanisms. These mechanisms involve many genes and regulatory factors related to a network that causes different expression patterns of genes under different conditions. This study investigated the expression pattern of one transcription factor gene, WRKY29 under both biotic and abiotic stress conditions. Stresses include *Heterodera filipjevi*, *Fusarium culmorum*, and drought which were applied individually, double, and triple simultaneously. It was found that drought as abiotic stress is the main factor in increasing the expression level of *WRKY*29 gene. One week after double simultaneous stress drought plus *Heterodera* in 'Silverstar' (resistant to *Heterodera*) accession, the highest gene expression was obtained. But the level of expression decreased in triple simultaneous stress (drought+*Heterodera*+*Fusarium*) which caused damage and reduced the yield components traits. The level of gene expression was reduced under triple stress because of the antagonistic effect of fungus on nematodes and different pathways of fungal resistance and drought tolerance in wheat. This accession was susceptible to fungal stress and had the lowest gene expression under this stress. Accession 2–49 (resistant to *Fusarium*), the highest gene expression was in fungal stress. Since 2–49 was known as a drought-resistant accession, the WRKY gene expression was also high in drought stress. However, different stress combinations cause unpredictable plant responses, by studying the pattern expression of genes involved in resistance pathways, the potential of the plant can be understood generally.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops for global food security that is exposed to different kinds of stresses. The diversity of stresses, resistance mechanisms, and genes involved in resistance pathways, made us study the gene expression pattern of the *WRKY* gene family under stress conditions in wheat. Naturally, plants are damaged by multiple types of stresses. Different biotic and abiotic factors with various intensities occur during plants' growth and affect their yield and survival rate. Plants have a complicated response to these stresses including numerous physiological and molecular reactions. Many genes and regulatory elements are involved in the plant resistance and tolerance pathways (Ahmadi *et al*. 2022). Different environmental stress interactions elicit an exclusive complex cellular and molecular response. In this way, different gene expression patterns can be created. WRKY transcription factors (TFs) consist of a large gene family, spread widely in plants. These genes have key roles in the regulation of plant growth, development, metabolism, and responses to biotic and abiotic stresses (Chen et al. 2019). WRKY TFs, DNA binding proteins, have a positive and negative regulation effect on gene expression by binding to the W-box (TTGACC/T) sequence in promoters (Huang et al. 2019). In wheat, 174 WRKY proteins are identified (Ning et al. 2017).

It is important to study the effect of simultaneous stresses because of the complexity of the plant reaction in different conditions for example co-occurrence of CCNs and root rot fungi may cause more yield loss and root damage than the occurrence of each pathogen alone (Hassan et al. 2012), Cereal cyst nematodes reduce water uptake and nutrient flow by attacking host plant roots and intensify the drought stress effect (Nicol 2002), or first pathogen attack may induce plant defense response and change their basal susceptibility to other pathogens and increase defense ability as well as drought tolerance (Van Hulten et al. 2006). Also, an antagonistic interaction can occur between fungi and nematodes where the fungus reduces the hatching of nematode cysts and eggs, thereby reducing the invasion of plants by the juveniles (Hassan et al. 2012, Ahmadi et al. 2022).

Therefore, the main objective of this study is to investigate the expression pattern a transcription factor gene, WRKY29, under both biotic and abiotic stress conditions.

Methods

Plant materials and stress treatments: Two wheat accessions were studied for gene expression analysis. These accessions were 2–49 (resistant to *Fusarium*) and 'Silverstar' (resistant to *Heterodera*). Through primary studies by the Soil Borne Pathogens program at the CIMMYT-Türkiye office, a set of wheat germplasm with differing levels of resistance/ tolerance to CCN, crown rot, and drought was treated by *H. filipjevi, F. culmorum*, and drought, independently and simultaneously (Ahmadi et al. 2022).

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Experimental design and sampling: Sampling of wheat root tissue, for RNA extraction and gene expression analysis, was performed three times after exerting stress conditions. These three sampling times are important stages in the early transcriptional response of plants to stress (Kong et al. 2015). Sampling subtended six replicates (two biological and three technical). Each biological replicate sample contained five seedlings.

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RNA extraction, cDNA synthesis, and qRT-PCR: Total RNA was extracted from cleaned root tissues, using RNA Isolation Kit (S-1010 DENA list ASIA). Thermo Company DNase was treated to remove DNA contamination from the extracted RNA samples. To verify the concentration and purity of extracted RNA, using the ratio of absorbance at 230 nm, 260 nm, and 280 nm in a spectrophotometer. Single-stranded cDNA synthesis was done using PrimeScript^{TM} RT reagent Kit from Takara, following the manufacturer's guidelines to design gene-specific primers, the gene sequence of transcription factors was obtained from https://www.ncbi.nlm.ni,h.gov, and primers were designed by online software (http://www.bioinformatics.nl/cgibin/primer3plus/primer3plus.cgi). Quantitative real-time PCR analysis was performed on a StepOnePlus Real-Time PCR System (Rotor-GeneQ, QIAGEN Company).

Results

Gene identification: Recent studies have indicated that WRKYs, one of the largest families of regulators, are very important components of the plant signaling web. These DNA-binding transcription factors regulate specific transcriptional pathways during plant growth, development, and defense response to a variety of biotic and abiotic stimuli (Ahuja *et al*. 2010, Bakshi and Oelmüller 2014, Rushton et al. 2012).

The obtained results identified *WRKY*29 gene seems to be involved in transcriptional response to stress factors. There are one WRKY domain and one zinc-finger-like motif in each of these proteins. Based on the structure of their zinc-finger-like motif, *WRKY29* belongs to group III. *WRKY*29 gene, located on wheat chromosome arm 2BS, produced protein has 293 amino acids with 31.1185 KD molecular weight. This protein includes conserved domain WRKYGQK and C2HC zinc-finger-like motif (C-X7-C-X23-HTC) (Ning et al. 2017).

Gene expression analysis: The obtained results showed that the relative expression level of the three targeted gene increased in all stress conditions during the three sampling times in both accessions; 2–49 (resistant to *Fusarium*) and 'Silverstar' (resistant to *Heterodera*), and the highest gene expression level was obtained in 'Silverstar'. Generally, the rate of relative gene expression was incremental and reached the maximum level after one week.

Drought stress caused the most intensive reactions *WRKY29* gene. Nematode was the second factor to induce *WRKY* gene expression. Overall, the relative gene expression level for *WRKY29* gene in 'Silverstar' was statistically higher than 2–49 accession. At the same time, the expression level of *WRKY29* in 'Silverstar' (resistant to *Heterodera*) were higher than the expression level in 2-49 accession against nematode invasion, but against fungal attack, the expression level of gene in 2–49 (resistant to *Fusarium*) was higher than 'Silverstar' and caused early resistance in these accessions.

Analysis of variance showed that the main effects of drought stress, nematode stress, sampling time, and genotype cause significant changes in the expression level of gene *WRKY29*. But the effects of double and triple simultaneous stresses did not differ significantly. Triple simultaneous stress conditions increased the gene expression levels not more than others.

Discussion

Wheat is an essential global food crop. Considering its importance, efforts have been made to identify the entire genome of wheat. Genomic and transcriptomic resources of wheat were used to study the *WRKY* gene family. Earlier studies have confirmed that the WRKY transcription factor plays a fundamental role in regulating different physiological processes, such as plant growth, development, plant senescence, signal molecule delivery, biotic or abiotic stress responses, and synthesis of resulting metabolites (Wang et al. 2021).

Many studies show that a large portion of *WRKY* genes is responsive to pathogens, elicitors, and defense-related phytohormones such as salicylic acid (SA) or jasmonic acid (JA), implying a major role for the *WRKY* gene family in plant immunity. WRKY TFs also regulate plant response to stress through abscisic acid (ABA) and ROS-related signaling pathways. During drought stress, the amount of ABA is increased in plants, and to reduce transpiration rate leaf stomata are closed, thus regulating water balance in plants (Wang et al. 2021).

Drought stress is the main reason in the world that reduces wheat production significantly (Keating et al. 2014). Preservation of grain yield under water stress can be achieved first by maximizing soil water capture through the root system (Blum 2009). Many root pathogens, like nematodes, use the xylem of root to obtain nutrient material, this can disturb water transport and cause water-deficient stress (Stirling and Stanton 1997). Co-occurrence of drought and nematode stress can exacerbate the effects of water scarcity. So, any drought stress tolerance mechanism also could be effective for nematode stress resistance too. Second, grain-filling, an important process in yield formation, is mainly sustained by the photosynthesis of flag leaves and spikes under drought treatment (Jia et al. 2015). It is shown that different *TaWRKY* genes are preferentially expressed in specific tissue during the grain-filling stage under water-deficit conditions.

As found in this study drought stress was the most effective factor in increasing the expression level of *WRKY* gene. Since this up-regulation occurred highly in the resistant genotype (Ahmadi et al. 2022) it can be concluded that this gene has a positive effect on regulating the expression of drought tolerance genes and is up-regulated under abiotic stress conditions. Due to the nematode stress that can increase the severity of drought stress, the highest level of gene expression was obtained at the simultaneous double stress drought/*Heterodera* condition.

Acknowledgments

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Status of plant parasitic nematodes in some maize fields in Iran

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Summary

Maize (*Zea mays* L.) is one of the most important staple crops. The main maize producing regions were subjected for sampling to survey the plant parasitic nematodes, as many as 216 soil and root samples were collected from Fars, Ghazvin, Kermanshah, Khuzestan and Tehran provinces during 2017 to 2021. The samples were examined and the nematodes were studied by morphological and morphometric characters, occasionally molecular aspects. The results showed among the identified species, root lesion nematodes (RLN) species possessed the maximum population density and frequency (54%) as the most dominant parasitic nematodes in root and soil samples in maize fields (78%, 55%, 53%, 50% and 32% abundance in maize fields of Qazvin, Tehran, Kermanshah, Khuzestan and Fars provinces, respectively). *Pratylenchus* mediterraneus was found in a maize field in Andimeshk, Khuzestan, the sequences of the partial 28s-rRNA, D2-D3 expansion region was deposited in the GenBank database under the accession number MW959145. To our knowledge, it is the first report of maize infection by *P.* meditrraneus in the world. Cereal cyst nematodes had 3- 40% abundance in maize fields of the studied provinces.

The incidence of polyphagous, migratory endoparasites of two devastating genera of root lesion nematodes, *Pratylenchus* and *Pratylenchoides* in the main maize producing provinces in Iran, definitely requires much attention and as a threat in maize production needs intensive studies.

Introduction

Maize, rice and wheat are the major staple food crops in the world. Maize is one of the most important and high yielding grains with nutritional importance, which has the highest production among the three mentioned cereals. Maize hosts a large number of pathogens such as fungi, bacteria, viruses and plant parasites nematodes (PPNs). Over 120 nematode species were reported in association with maize in different regions of the world, which root knot nematodes (*Meloidogyne* spp.), Root lesion nematodes (*Pratylenchus* spp.) and cyst nematodes (*Heterodera* spp.) are addressed as the major parasitic groups. Several studies have been reported the most common PPNs genera associated with maize as *Pratylenchus*, *Helicotylenchus*, *Xiphinema*, *Ditylenchus*, *Meloidogyne*, *Criconemella*, *Heterodera*, *Paratrichodorus*, *Rotylenchulus*, *Scutellonema*, *Longidorus*, *Radophulus*, *Trichodorus*, *Tylenchorhynchus*, *Globodera*, *Hemicriconemoides*, *Haplolaimus* and *Scutellonema* in the world (CABI 2020).

Various PPNs were sporadically reported from maize fields in Iran which belonged generally to the family of Tylenchidae Örley, 1880, subfamily Merliniinae Siddiqi, 1971 and Pratylenchidae Thorne, 1949, mostly from soil around the roots and rhizosphere (Kheiri 1972, Niknam and Kheiri 1997).

Regarding the importance of maize cultivation in Iranand the study of biotic agents involved in the damage and crop reduction, the incidence of plant parasitic nematodes was surveyed amid a national project in five main maize producing regions, *i.e*. Tehran, Khuzestan, Qazvin, Fars and Kermanshah in Iran.

Methods

A total of 216 composite root and soil samples were collected from the maize fields of Fars, Ghazvin, Kermanshah, Khuzestan and Tehran provinces. The samples were processed by the centrifugal flotation and Whitehead tray method (Whitehead and Hemming 1965). The abundance of plant parasitic nematodes was numerated in the extracted nematode suspension under a microscope. For morphological studies, the nematodes were fixed then transferred to dehydrated glycerin for mounting. The morphometric features were measured using an Olympus BH-2 microscope. For some molecular studies the total DNA were extracted from 4-5 females through worm lysis buffer, the procedure for PCR and sequencing were followed the protocol described by Tanha Maafi et al. (2003). The D2-D3 expansion segments of the 28S rRNA gene were amplified with the forward D2A and the reverse D3B primers (Subbotin et al. 2006).

Results and discussion

In this study the following genera and species of plant parasitic nematodes were identified: *Amplimerlinus* sp., *Aphelenchoides* sp., *Boleodorus tylactus*, *Ditylenchus myceliophagus*, *Ditylenchus* sp., *Filenchus* sp., *Geocenamus rugosus*, *Helicotylenchus digonicus*, *Helicotylenchus vulgaris*, *Heterodera avenae*, *H. filipjevi*, *H. latipons*, *Merlinus brevidens*, *Neopsilenchus* sp., *Paratylrnchus arculatus*, *Paratylrnchus* sp., *Pratylenchoides ritteri*, *Pratylenchus* mediterraneus, *Pratylenchus neglectus*, *Pratylenchus thornei*, *Scutylenchus rogusus*, *Tylenchorhynchus* sp.

The results showed root lesion nematodes (RLN) species possessed the maximum population density and frequency (average 54%) as the most dominant nematodes in root and soil samples in maize fields. Their abundance was determined 78%, 55%, 53%, 50% and 32% in maize fields of Qazvin, Tehran, Kermanshah, Khuzestan and Fars provinces, respectively.

Pratylenchus mediterraneus was found in a maize field in Andimeshk, Kuzestan. To our knowledge, it is the first report of maize infection by *P.* mediterraneus in the world. The sequence of the partial 28s D2-D3 region of *P.* mediterraneus was deposited in the GenBank database under the accession number MW959145. According to references, root lesion nematodes are one of the main destructive agents causing yield loss in maize, as well as their contribution for the predisposition of the host plant to invasion with other soil borne pathogens indicates more destructive role for their pathogenicity in crops (McDonald and Nicol 2005; Kimenju et al. 1998).

In addition to the RLNs, species of cereal cyst nematodes, *Heterodera avenae* group were also found in the soil samples showing 40%, 32%, 22%, 7% and 3% abundance in maize fields of Tehran, Fars, Khuzestan, Qazvin and Kermanshah provinces, respectively. Presence of CCN group in corn fields implies two principal important points, the rotation program which maize is a rotational crop with wheat for wheat growers in the surveyed areas, and the second assumption the potential pathogenicity of these nematodes on this grain crop that needs intensive research.

Pratylenchoides ritteri close to *Pratylenchus* with paired genital tracts belonging to subfamily Radopholinae Allen & Sher, 1967 was only identified from the samples collected from Kermanshah province where the species is widely distributed in wheat farms, and frequently reported as a major parasite of wheat from this region. The occurrence and host suitability of *P. ritteri* on maize has already been studied in some corn fields in northern Iran, Mazandaran province as the first report of its damage on maize (Majd Taheri et al. 2013).

The incidence of polyphagous, migratory endoparasites of two devastating genera of root lesion nematodes, *Pratylenchus* and *Pratylenchoides* in the main maize producing regions in Iran, definitely requires much attention and as a threat in maize production needs intensive studies.

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Biodiversity of nematode communities associated with wheat (*Triticum aestivum* L.) in southern Morocco and their contribution as soil health bioindicators

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Summary

Soil nematodes are major soil organisms known to reduce wheat production worldwide. However, they are a crucial part of soil biodiversity. Due to the limited data available regarding nematode biodiversity associated with cereal crops in Morocco, a survey was conducted in 2019 across 80 wheat fields. Thirty–three nematode taxa were obtained with different trophic groups: 11 bacterivores (i.e., *Rhabditis* and *Cephalobus* spp.), 11 herbivores (i.e., *Pratylenchus* and *Heterodera* spp.), 6 fungivores (i.e., *Ditylenchus* and *Aphelenchoides* spp.), 4 omnivores (i.e., *Dorylaimus* and *Aporcelaimus* spp.) and one predator (*Mononchus* spp.). For each locality, the diversity of nematode communities was assessed through multiple diversity indices. Three areas (Ait Melloul, Oulad Dahou, and Sidi Boushab) were more diversified and had pronounced nematode occurrence. Moreover, few localities (e.g., Ait Amira and Lqliaa) were shown to be disturbed. Our study underlined that several nematode taxa were significantly correlated with edaphic and climatic/geographic properties. On the other hand, the spatial and parsimony analyses revealed that plant feeders were most associated with bacterial feeders in disturbed soils. This work revealed that the soil nematodes associated with wheat crops are diverse and can serve as an important tool for soil biomonitoring at a large scale.

Introduction

Wheat (*Triticum* spp.) is the most widely grown crop in the world, with more than 218 million ha, and its world trade is much greater than for all other crops combined (FAOSTAT 2020). Morocco is the world's 23rd wheat-producing country. Wheat is cultivated over 3.2 million ha and about 8 million tonnes were produced in the 2019–20 growing season including 2.2 million ha of bread wheat and 1 million ha of durum wheat (FAOSTAT 2020). Plant-parasitic nematodes (PPNs) are one of the main biotic factors that attack and damage plant roots causing plant stress, ultimately leading to a significant yield loss (Dababat and Fourie 2018). In cereals, PPNs mostly belong to three groups, viz., cereal cyst nematodes (CCN, *Heterodera* spp.), root-lesion nematodes (RLN, *Pratylenchus* spp.), and root-knot nematodes (RKN, *Meloidogyne* spp.). These nematodes are distributed globally and implicated in significant economic yield loss (Dababat and Fourie 2018). Diversity and soil-related functions have recently led to the concept of "soil quality" defined by its ability to function within a given ecosystem to sustain organic production, maintain environmental quality, and promote plant health (Doran 2002). The knowledge of the structure of the nematode community provides information related to the different processes performed in the soil, the food web in the soil, and the state of stability of agro-ecosystems and soil biodiversity (Bongers 1990; Villenave el al. 2009). The set of nematode characteristics as bio-indicators of agricultural soils is relevant for the study of environmental changes (Ferris et al. 2001).

In Morocco, most research has focused solely on PPNs communities, and to our knowledge, data on other nematode communities and their use as bio-indicators for assessing soil quality have not been reported yet. Therefore, the objectives of this study were to (i) investigate all soil nematode communities in South Moroccan wheat agro-systems, (ii) evaluate the potential of nematodes as potential bioindicators of soil quality and health in wheat fields, and (iii) predict the spatial contribution and succession of each nematode trophic levels in regards of soil health attributes.

Methods

Nematode Sampling Design: An intensive survey was established to investigate the diversity of nematode communities in different wheat agro-systems in the Southern regions of Morocco. The surveyed fields were selected based on their contribution to total production, soil type, and geographic distribution. Samples were taken during the wheat heading growth stage from 10 provinces accounting for 80 representative fields.

Nematode Processing: Nematode extraction was carried out via a modified Baermann technique for both soil (100 cm^3) and root (20 g) matrices. Nematode suspension was collected and processed under a stereomicroscope (Olympus CH–2; Japan) after 72 h of incubation. Nematodes were killed and fixed. Soil nematodes were identified to genus level using dichotomous keys based on morphological features including body traits, stylet type, lip region, head shape, pharyngeal overlap, tail type, spermatheca shape, and vulva position. Species of root-knot nematodes (RKNs) were identified via the establishment of the perineal patterns. For cereal cyst nematodes (CCNs), vulval cones were established for identification.

Soil Physico-Chemical Analyses: Soil physico-chemical analyses were performed at the soil laboratory of INRA–Agadir, using regular methods. Soil texture proprieties including clay (0–2 µm), silt (2–50 µm), sand (50 to >200 µm), and Limestone proportions were thoroughly analyzed. The electrical conductivity (µS/cm) and pH were determined via the 1:2.5 soil: water ratio technique. As for the mineral content, the rates of potassium (K), manganese (Mn), and magnesium (Mg) alongside the exchangeable acidity were distinguished via atomic absorption spectrometry.

Diversity Assessment of Nematodes Taxa: Nematode incidence was determined by determining the prevalence and maximum density for each nematode feeding type. For more advanced biodiversity screening, several ecological and biomonitoring indices were measured to assess nematode diversity and structure in each locality surveyed. These indices included Shannon–Weaver diversity index (H'), maturity index (MI), plant-parasitic index (PPI), channel index (CI), Enrichment index (EI), and structure index (SI).

Statistical Analyses: To explore nematodes' community and soil physical-chemical patterns from different wheat agro-systems surveyed, principal component analyses (PCA) were conducted. Food web and metabolic footprints analysis was conducted using NINJA (Nematode INdicator Joint Analysis) software. Advanced heatmap analysis (heatmap-2) was adopted to visualize correlations between nematodes and soil physical-chemical properties, based on Ward's algorithm of R software. Nematode spatial contribution and parsimony mapping network (PMN) analyses were conducted using BioPython matplotlib library.

Results and discussion

The obtained results showed that 33 nematode genera were identified from this survey. Plant-parasitic nematodes (herbivores) were accurately represented in all localities with 40% of occurrence, followed by bacterivores (30%), fungivores (20%), both omnivores and predators (10%). The maturity index (MI), which focuses on free-living taxa was significantly different between the localities surveyed. The channel index (CI), which implies the decomposition aspects of both fungivorous and bacterivorous nematodes showed significant differences between wheat fields from different areas. It was indicated by the heatmap analysis that all nematode taxa identified were significantly correlated with calcium, Mn content, and total organic matter (Tom). Interestingly, soil texture patterns (sand, clay, and silt content) were also positively correlated with many soil nematode taxa.

The Shannon–Weaver diversity index was significantly ($P < 0.05$) higher in Oulad Dahou, Ait Melloul, and Sidi Boushab (2.91, 2.7, and 2.49, respectively), compared to the other localities. The lowest index value was reported in Ait Amira province (1.2). There was no significant difference in evenness between all localities. The potential of nematodes as effective soil health bio-indicators in wheat fields was assessed using food web diagnostics. Four localities (Massa, Sidi Bibi, Belfaa, and Ait Melloul) were distinctively highly enriched and matured with a high abundance of bacterial feeders. Furthermore, fertile soils were accurately presented in Oulad Dahou, Temsia, and Biougra provinces. Soil disturbance in wheat fields was highlighted mainly in Ait Amira, Laqliaa, and Sidi Boushab provinces (Figure 1).

For a better understanding of soil health bioindication potential embodied within soil nematodes, spatial contribution analysis was conducted via projecting the ANOSIM and SIMPER analyses outputs. The succession of nematode functional guilds (FGs) and trophic levels (TLs) was channeled through a machine learning-related analysis for a better understanding of its affiliation with soil disturbance attributes observed throughout the surveyed wheat regions (Figure 2). The Venn diagram depicts the possible combinations that might occur in soils. Successions started with plant feeders (PFs) were shown to be consistently associated with fungal feeders (FFs) (WSRP = 60%; $P < 0.001$) compared to other TLs linkages. Furthermore, bacterial feeders (BFs) had prominent relationship with omnivore organisms (Os) (WSRP = 50%; $p < 0.001$). Tripartite successions were not much anticipated in the sense that low WSRP values were recorded. On the other hand, 30% of all FGs (PF–FF–BF–O–P) have occurred in the same frame (soil or rhizosphere platforms).

Climatic or geographic factors can be a crucial indicator of many plant-related organisms' distribution, such as nematodes. Redundancy analysis (RDA) was conducted to evaluate the relationship between nematode trophic levels represented by several taxa identified and climatic/geographic factors attributed as MAP (mean annual precipitation), MAT (mean annual temperature), LA (latitude), and LG (longitude). Across all localities studied, multiple correlations were detected. For instance, plant parasites (PP) were significantly related to the MAP patterns ($P < 0.01$), while bacterivore and fungivore nematodes were mostly dependent on MAT ($P < 0.01$ and $P < 0.05$, respectively) (Figure 3). Moreover, geographical attributes (latitude and longitude) were predicted to be affecting omnivores and predators, respectively. Nielsen et al. (2014) reported that the rise of nematode abundance might be due to an increase in annual temperatures and Imren et al. (2017).

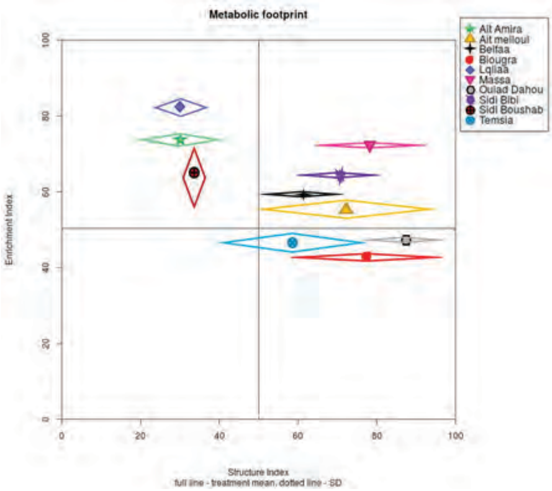

Figure 1. Nematode food web diagnostics and their position as soil health bioindicators.

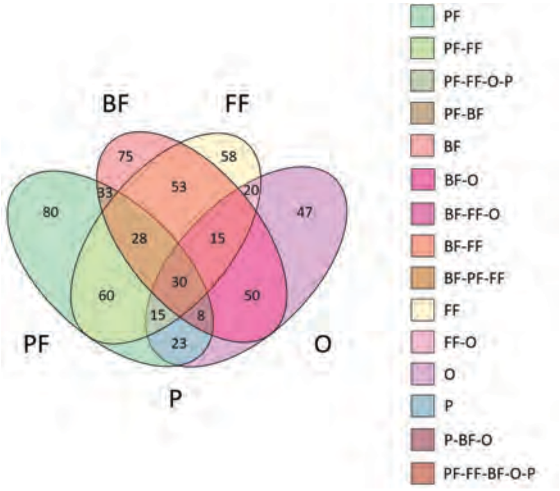

Figure 2. Functional guilds and trophic levels succession of soil nematodes on soil disturbance attributes in wheat fields. Venn diagram depicting all nematode trophic levels successions. The numbers represent the weighted species richness percentage (WSRP) calculated in all surveyed areas. Abbreviations stand for: PF Plant Feeders, FF Fungal Feeders, BF Bacterial Feeders, P Predators, and O Omnivores. The plots were generated using BioPython matplotlib library via Matplotlib-venn module in PyCharm API.

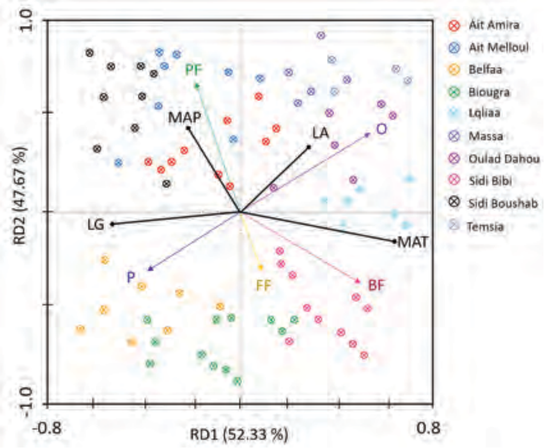

Figure 3. Redundancy analysis (RDA) of soil nematode taxa and environmental factor variables (LA—latitude; LG—longitude; MAP—mean annual precipitation; MAT—mean annual temperature). Soil nematodes are grouped into 4 trophic guilds (BF —bacterial feeders, FF—fungal feeders, PF—plant feeders, O—omnivores, and P—predators); Crossed circles indicate sampling localities; the bold black lines indicate environmental factors. The plot was generated by Python matplotlib and ordination libraries (skbio.stats.ordination).

To sum up, this study provides new insights into genera diversity, incidence, and distribution of soil nematodes associated with the wheat crop in southern Morocco, with their contribution as potential bio-indicators for soil health aspects. Nematodes from different trophic groups were differently distributed in all localities surveyed. Herbivorous taxa dominated by Pratylenchidae and Heteroderidae families alongside bacterivores represented mainly by Rhabditidae and Cephalobidae families were the most pronounced. Soils from Ait Melloul, Belfaa, Oulad Dahou, and Sidi Boushab were more diversified. On the other hand, few localities (e.g., Ait Amira and Lqliaa) were shown to be disturbed. Therefore, an equilibrium between taxa was observed in all surveyed areas. Possible soil quality patterns were thoroughly analyzed, and soil nematodes proved to be promising indicators. To our knowledge, the explicit spatial relationship of all nematode taxa from different functional guilds predicted by multidimensional contribution analysis is the first to be conducted and will add a needed finding to parasitic–saprophytic interactions. In addition, the soil networking embodied within microorganisms puts nematodes as the main actors for many biodiversity-related patterns. Due to climate change, the within soil nematode communities tend to fluctuate due to many factors (temperature and precipitation), which significantly affect soil health. More research is required to expand survey areas in all Moroccan cereal growing areas to identify all nematode taxa at the species level and study the possible nematode communities' responses to agricultural practices and environmental constraints for a better understanding of their role as soil health bioindicators. This will be helpful to farmers to establish adequate management and cereal soil processing strategies against potentially related pests.

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Identification of wheat germplasm for cereal cyst nematode (*Heterodera filipjevi*)

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Summary

A winter wheat set provided from wheat breeding program of the Transitional Zone Agricultural Research Institute (TZARI), Eskisehir were evaluated for their resistance against cereal cyst nematode (*Heterodera filipjevi*) under the controlled growthroom conditions. This set comprised of the following nurseries; Stem rust nursery (SRN #76), Leaf rust nursery (LRN #27), Durum stem rust nursery (DSRN #27). One hundred thirty germplasm were screened for *H. filipjevi* as 3 reps in complete randomized block design. The grouping was performed based on cyst number per plant compared to that cyst numbers on the known checks. Out of the 130 germplasm screened, 21 wheat germplasm showed promising resistance for cereal cyst nematode-*Heterodera filipjevi* under growth room conditions). Out of the 21 germplasm selected 7 was from durum stem rust nursery, 5 from leaf rust nursery, 9 from stem rust nursery.

Introduction

Cereal cyst nematodes (CCNs) are among the most important nematode pests that limit production of small grain cereals (Smiley et al. 2017*). Heterodera filipjevi* has been recorded in many countries such as China, Estonia, India, Iran, Libya, Morocco, Norway, Pakistan, Russia, Sweden, Tadzhikistan, Tunisia, Türkiye and USA (Rumpenhorst et al. 1996; Holgado et al. 2004; Smiley et al. 2008; Riley et al. 2009; Nicol et al. 2011). The cereal cyst nematodes (CCNs) are a group of closely related species that have been documented to cause economic yield losses, especially in non-irrigated wheat production systems in North Africa, West Asia, China, India, Australia, the United States, and parts of Europe (Nicol and Rivoal, 2008). Losses in wheat yield in research trials of up to 20, 50, 50, and 90%have been reported from Pakistan, Australia, Türkiye, and Saudi Arabia, respectively (Imren et al. 2015; Riley and McKay 2009). One of the most effective strategy is to use resistant germplasm for cereal cyst nematodes, if available. Crop rotation, different tillage techniques are offered to control cereal cyst nematodes. In this study a collection of wheat lines also good for rust diseases have been tested for *Heterodera filipjevi* for genetic resistance.

Methods

A total of 130 wheat germplasm (Table 1) with good rust resistance background have been screened for cereal cyst nematode (*Heterodera filipjevi*) under controlled growth room conditions at the Transitional Zone Agricultural Research Institute (TZARI) in Eskisehir-Türkiye. The wheat germplasm provided by TZARI-Eskisehir breeding program.

Table 1. List of nurseries screened for cereal cyst nematodes (*Heterodera filipjevi*)

Nursery Name	Type	#
Stem Rust	BW	76
Leaf Rust	BW	27
Durum Stem Rust	DW	27

Nematode Inoculum: Screening tests were carried out using a population of H. *filipjevi* collected from naturally infested field in Kırsehir, Türkiye (39° 39′ 709″ N, 32° 37′ 14″ E) (Peng et al. 2013).

Growth Room Screening: Single pregerminated wheat seed was planted in standard small tubes (16 cm in height × 2.5 cm in diameter) containing sterilized mixture of sand, field soil, and organic matter (70:29:1 v/v). The field soil and sand were sieved and sterilized at 110 °C for 2 h for 2 successive days, and the organic matter was kept at 70 °C for 5 h. At the sowing date freshly hatched 300 juvenile/ml were inoculated on the soil around the stem base. The plants were grown in a growth chamber with a 16-h artificial photoperiod and maintained at a temperature of 22 ± 3 °C with 70% relative humidity.

Experimental Design: Each treatment was replicated 3 times and tubes were placed in a completely randomised block design.

Harvest: The plants were harvested 9 weeks after juvenile inoculation. Soil from each tube was collected in a 2-L pot filled with water for cyst extraction, while roots were washed on nested sieves with 850 µm and 250 µm mesh sizes to free cysts from the root system. Cysts from both root and soil extractions were collected on the 250-µm sieve and counted under a stereomicroscope.

Assessment: The grouping was performed based on cyst number per plant compared to that cyst numbers on the known checks. The following ranking was followed; 1= resistant (R), 2= moderately resistant (MR), 3= moderately susceptible (MS), 4= susceptible (S) and 5= highly susceptible (HS) (Dababat et al., 2019).

Results

The cyst numbers ranged from 1 to 36 and 1 to 38 in bread wheat and durum wheat, respectively. A total of 21 wheat germplasm showed promising resistance for cereal cyst nematode – *Heterodera filipjevi* under growth room conditions (Table 2). Five germplasm found Resistant (R) (3.8%), 16 Moderately Resistant (MR) (12.3%), 42 Moderately Susceptible (MS) (32.3%), Susceptible (S) 53 (40.8%) S and 14 (10.8) Highly Susceptible (HS). Out of the 21 germplasm selected 7 was from durum stem rust nursery, 5 from leaf rust nursery, 9 from stem rust nursery.

Discussion

Use of host-plant resistance and tolerance against CCNs is one of the most cost effective and prominent approaches to minimize crop losses below the threshold level (Trudgill et al.,1991). Wheat germplasm with good resistance to the cereal cyt nematode (*Heterodera filipjevi*) in combination with high yield is

Table 2. List of the best performed germplasm in terms of resistance for cereal cyst nematodes (*Heterodera filipjevi*)

SBP No	Nurs	Pedigree	Mean Cyst No	Grouping
2	SRN	BYE*2/TC//KIZILTAN/C1252 (KIRMIZI BASAK)/3/MIRZABEY	2.2	R
3	SRN	BYE*2/TC//KIZILTAN/C1252 (KIRMIZI BASAK)/3/EMINBEY	3	R
5	SRN	KHSPDK-33/SOYER	1.8	MR
27	SRN	ICEBERG/DF142-85//ANK-98	2.7	R
34	SRN	PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/ YR/4/TRAP#1/6/SW89-5124*2/FASAN//SHAAN 229(OCW02S623S-2)/7/N44/OK94P455(OK03716W)	5.2	MR
36	SRN	ZENCİRCİ-2002/NİKONOVAKA//TOSUNBEY	5	MR
37	SRN	SOYER02/4/TAM200/KAUZ/3/ES14/SITTA//AGRI/NAC	4.5	MR
38	SRN	SONMEZ01/F06325G1-2	3.7	MR
40	SRN	ZF/LDS//FAT/3/61-130/LDS/4/AU107/5/GDO/6/12IDYN-38	5	MR
81	LRN	SONMEZ01/IG-139883 (12SUNNPest RN-1)	5.2	MR
89	LRN	11MVD-55/LOUKOS3	5	MR
91	LRN	AKBASAK/BOTNO//KIZILTAN/3/KIZILTAN/4/ANK-98	2.8	R
92	LRN	ICEBERG/DF142-85//ANK-98	4.2	MR
96	LRN	MvM/Sana//Pfau/Mılan/3/Bul1518-4-38/4/BBVD(2011)-8	4.8	MR
107	DSRN	ESPERYA/PEHLİVAN	4.2	MR
108	DSRN	HARMANKAYA99/ST.ERYHTR1290-08(18FAWWON-IR-115)//00KE3	2.5	MR
113	DSRN	61-130/TELEMARA//UNKNOWN/3/12IDYN-27	4.7	MR
120	DSRN	ZF/LDS//FAT/3/61-130/LDS/4/AU107/5/GDO/6/12IDYN-38	5	MR
121	DSRN	AKBASAK/BOTNO//KIZILTAN/3/KIZILTAN/4/ANK-98	2.5	R
124	DSRN	YERLİ54"S2"/4/ÜVY162/61-130//KOBAK 2916/LDS/3/SİN-CAPOG/5/ZENIT	4.8	MR
125	DSRN	Beliava x Çetinel	4.8	MR
		Katea (*Resistance check*)	2.8	R
		Sonmez (*Resistance check*)	5.7	MR
		Kutluk (*Susceptible check*)	15.5	MS
		Bezostaja (*Susceptible check*)	26.7	S

preferential for breeding purpose. The purpose in this study was to explore new sources of resistance germplasm in wheat for *H.filipjevi*. The selected germplasm might be tested under open field conditions and used for crosses in breeding programs.

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Discovery and distribution of root lesion nematode *Pratylenchus neglectus* in wheat fields in Pakistan

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Summary

The root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) are major pathogens of cereals in many regions worldwide. Wheat genotypes resistant to these nematodes can be determined from final nematode population densities in controlled environmental chambers or glass house. To assess the prevalence of root lesion nematodes in wheat experimental field area of National Agricultural Research Center, Islamabad, soil samples along with wheat seedlings of fifty-seven historical wheat genotypes were collected in 2022. The nematode species *P. neglectus* was found in all the wheat genotypes associated with roots as well as in root rhizosphere. For fields having root lesion nematodes in 2022, *P. neglectus* mean population densities were found in Margalla variety with maximum nematode population (2033/100gr) of soil, followed by wheat varieties as Markaz-19 (1446/100grams), Bakhtawar (1320/100gr), Suleman-96 (1140/100grams), Johar-16 (1053/100grams), Pastor (1040/100grams), Zinkol-2016, a zinc fortified wheat variety (1026/100grams) and WL711 (1000/100grams). Stunt nematodes (*Tylenchorhynchus* spp.) were also detected from all the root rhizosphere in association with the trial.

Introduction

Among the plant parasitic nematodes, the root lesion nematode (*Pratylenchus thornei*) is a major pathogen of wheat (*Triticum aestivum*) in different agro-ecologies of the world (Smiley and Nicol, 2009). The family Pratylenchidae (Thorne 1949), is hosted by many cereal and pulse crops species. These nematodes cause losses to wheat genotypes up to 65% yield (Thompson et al. 1999). In dry land wheat production systems of the world, two species of root lesion nematode (*Pratylenchus thornei* Sher & Allen and *P. neglectus* (Rensch) Filipjev Shuurmans & Stekhoven) are causing serious damage to the wheat production annually (Smiley et al., 2004; Thompson et al., 2008). The estimated yield losses reported from these nematodes are about 85% in Australia, 50% in USA, and 35% in Mexico. These species infest wheat roots, resulting in reduced growth, along with establishment of dark lesions on roots. These lesions serve as secondary infection sites for other soil microorganisms (fungi, bacteria etc.) (Jones et al. 2013). The cereal crops infested while growing under moisture stress experience the maximum yield losses, however the above ground symptoms are usually non-specific and confused with nutrient deficiencies or drought stress. These include stunted growth, chlorosis, reduction in vigour, lower leaves death, grain yield, grain quality and their tiller count (Smiley and Nicol 2009; Doyle et al. 1987; Thopmson et al. 1995; Smiley 2005; Nicol and Ortiz-Monesterio 2004). Root lesions nematodes are completing their life cycle within the root system endoparasitically, consequently the greater population dynamics of *Pratylenchus* nematodes built up under annual wheat cropping systems (Williams et al. 2002; Smiley et al. 2005). In Pakistan, twenty species of family Pratylenchidae (Thorne 1949) encountered on various host plants and were recorded (Maqbool and Zarina 1988;Maqbool and Shahina 1988).

This aim of this study was preliminary observation of incidence, distribution, and association of root lesion nematodes (*Pratylenchus* spp.*)* with a historical set of wheat varieties in agroecologies of Pakistan. These wheat varieties were cultivated in different wheat agro ecologies.

Methods

All soil and wheat seedlings were taken after winter planting from December to March 2022. Three replicate samples were taken from each wheat variety to form a historical set. The bulked soil samples were placed in a soil collection bag and properly labeled accordingly. The soil samples were processed by Whitehead tray method (Whitehead and Hemmings 1965). For each soil sample, nematodes were extracted from 100 g of flesh soil over 48 h at 20 °C using 1 liter of tap water. Extracted nematodes populations were examined for the presence of root lesion nematodes (*Pratylenchus* spp.). The nematode suspension (2 ml) was placed into a counting dish and the nematodes were counted using stereomicroscope Nikon SMZ1.

Results and discussion

In 2022, *Pratylenchus* spp. were identified in all 57 soil samples of the wheat varietal historical set grown in agro ecology of NARC (Table 1). The population of *P. neglectus* was prominent in all the extracted nematodes from soil. The population densities were found in Margalla variety with maximum nematode population (2033/100 g) of soil, followed by Markaz-19 (1446/100 g), Bakhtawar (1320/100 g), Suleman-96 (1140/100 g), Johar-16 (1053/100 g), Pastor (1040/100 g), Zinkol-2016, a zinc fortified wheat variety (1026/100 g) and WL711 (1000/100 g) (Table 1). Stunt nematodes were also isolated and recovered from all samples examined among the nematode population.

Results of 2022 make up the first assessment of root lesion nematodes (*Pratylenchus* spp.) affecting wheat in the fields of NARC. *Pratylenchus* spp. were found primarily within the central winter wheat growing regions of Pakistan. These results will further be investigated for the next wheat crop in 2023 for the similar historical set or other wheat genotypes at NARC.

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Table 1. Nematode population density of root lesion nematodes in Wheat historical verities set cultivated in CDRI, NARC Pakistan

S.No	Varieties	Root lesion nematode /100 grams of soil	Nematode Identification (RLN)
1	Markaz 2019	1446	*P. neglectus*
2	Zincol 2016	1026	*P. neglectus*
3	Borlaug 2016	600	*P. neglectus*
4	Pak 13	800	*P. neglectus*
5	AzricDera	800	*P. neglectus*
6	NARC 2011	2033	*P. neglectus*
7	Narc 2009	680	*P. neglectus*
8	Margalla	653	*P. neglectus*
9	Wafaq	893	*P. neglectus*
10	Akbar 2019	273	*P. neglectus*
11	Ghazi	200	*P. neglectus*
12	Fakher Bhakkar	440	*P. neglectus*
13	Anaj 2017	593	*P. neglectus*
14	Ujala 16	1053	*P. neglectus*
15	Gold 16	433	*P. neglectus*
16	Johar 16	880	*P. neglectus*
17	Ahsan 16	620	*P. neglectus*
18	KPK 15	500	*P. neglectus*
19	Shahkar 13	840	*P. neglectus*
20	Galaxy 13	400	*P. neglectus*
21	Pirsabak 13	320	*P. neglectus*
22	Punjab 11	440	*P. neglectus*
23	AAS 11	820	*P. neglectus*
24	Millet 11	880	*P. neglectus*
25	Dharabi 11	600	*P. neglectus*
26	Faisalabad 08	960	*P. neglectus*
27	Meiraj 08	680	*P. neglectus*
28	Pirsabak 08	820	*P. neglectus*
29	Gandum 1	840	*P. neglectus*
30	Seher-06	720	*P. neglectus*
31	Fareed-06	940	*P. neglectus*
32	Pirsabak-05	640	*P. neglectus*
33	AAS-02	760	*P. neglectus*
34	Auqab	920	*P. neglectus*
35	Chakwal-50	1140	*P. neglectus*
36	M.H-97	1320	*P. neglectus*

37	Suleman-96	280	*P. neglectus*
38	Bakhtawar	540	*P. neglectus*
39	Pasban 90	960	*P. neglectus*
40	Ufaq	540	*P. neglectus*
41	Tatara	500	*P. neglectus*
42	Inqilab 91	440	*P. neglectus*
43	Barsat	840	*P. neglectus*
44	Maxipak	720	*P. neglectus*
45	Pak 81	840	*P. neglectus*
46	Khyber 87	540	*P. neglectus*
47	C-271	580	*P. neglectus*
48	C-273	920	*P. neglectus*
49	Paroula	340	*P. neglectus*
50	Pastor	720	*P. neglectus*
51	Bhakkar	1040	*P. neglectus*
52	Atta Habib	840	*P. neglectus*
53	Local White	1000	*P. neglectus*
54	WL 711	560	*P. neglectus*
55	Bahtor	700	*P. neglectus*
56	Dirk	820	*P. neglectus*
57	Pirsabak 08	440	*P. neglectus*

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Fusarium crown rot in southern Algeria on wheat: identification of associated species and assessment of aggressiveness

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Summary

Fusarium crown rot (FCR) is an important fungal disease of wheat worldwide. This research was carried out to study the most important species associated with this disease in Algeria. Symptomatic crowns were collected from different wheat growing regions in South Algeria. Morphological and molecular identifications showed the occurrence of seven fungal species isolated from crowns for the 3 years: *Fusarium culmorum* (25%), F. *pseudograminearum* (21%), *Microdochium* spp. (20%), *F. sporotrichioides* (15%) *F. avenaceum* (8%), *F. verticillioides* (6%) and *F. oxysporum* (5%). Sixteen isolates from symptomatic crowns were chosen for the pathogenicity tests. All strains revealed a significant difference in aggressiveness among the isolates. Results showed that *F. pseudograminearum* isolates were the most aggressive on the wheat seedlings.

Introduction

Fusarium crown rot causes browning of wheat seedling, brown discoloration of subcrown internodes and formation of bleached heads. Generally, the economic importance of the disease has been linked to environmental conditions. It has been recently reported that FCR may be responsible for as much as 50% production losses (Hollaway et al. 2013). Mostly, four pathogenic species including *F. pseudograminearum*, *F. culmorum*, *F. crookwellense* and *F. graminearum* have been reported as responsible for root rot and crown rot.

The severity of FCR is greater in dry soils and in areas with high temperatures (Scherm et al. 2013, Obanor and Chakraborty 2014, Balmas et al. 2015). These conditions are present in many wheat-growing regions in Algeria. It has also been reported that infection of the stem base of wheat by toxigenic *Fusarium* spp. leads to translocation of the toxin to the head and its accumulation in the grain (Covarelli et al. 2012, Beccari et al.2018b), suggesting FCR as a contributor to toxin contamination.

The objectives of this study were to identify the fungal species causing FCR of wheat in cereal regions in the South of Algeria, determine the frequency of each species, and assess the aggressiveness of the *Fusarium* isolates obtained from crowns

Methods

Surveys were carried in the end of March during three years in 2012, 2013 and 2014 to identify the species responsible for Fusarium crown root of wheat in 84 fields. Both durum (*Triticum turgidum* var. *durum*) and bread wheat (*Triticum aestivum*) were sampled in the main cereal regions in the south of Algeria. The plants collected were showing symptoms of the crown rot diseases.

Crown tissues were disinfected and analyzed. They were rinsed for 5 min in 2% sodium hypochlorite solution, rinsed three times in sterile distilled water then dried on sterile filter paper. The disinfested fragments were put on potato dextrose agar (PDA) and incubated at 22 °C for 5–7 days; subcultures from the margin colonies resembling to *Fusarium* or *Microdochium* were transferred on PDA medium. Single-spore cultures of the fungal strains were identified morphologically based on criteria cited by Leslie and Summerell (2006) and Hoshino et al. (2009). To confirm the morphological identification, molecular identification by use of species - specific primers was done.

For DNA extraction, fungal isolates were grown on potato dextrose agar medium (PDA) for 7 days. The mycelia were freeze-dried and ground to a fine powder. DNA was extracted by using a method adapted from Lamprecht et al. (1992). The DNA samples were amplified by PCR using the species-specific primers (Table 1).

Amplification products were separated by electrophoresis (80–90 V, 60 mA, 100 W, 30 min) in 1% agarose gels in 1X TAE buffer stained with 4 µl/ 100 ml of Clear Sight DNA Stain (Euromedex), and then photographed over a transluminator (UV BIORAD).

Table 1. Primer sequences used for PCR identification of *Fusarium* species

Species	Primers	Sequence 5'-3'	Tm	n	F (pb)
F. culmorum	**FC 01F[1]** **FC01 R[1]**	ATGGTGAACTCGTCGTGGC CCCTTCTTACGCCAATCTCG	60°C	35	570
F. pseudograminearum	**Fp1-1[3]** **Fp1-2[3]**	CGGGGTAGTTTCACATTTCYG GAGAATGTGATGASGACAATA	55°C	35	523
F. avenaceum	**JIAf[4]** **JIAr[4]**	GCTAATTCTTAACTTACTAGGGGCC CGTTAATAGGTTATTTACATGGGCG	60°C	40	220

Tm: temperature of hybridization for primers n: number of cycles F: size of fragment (kb)[1] Nicholson *et al*. 1998; [3] Aoki et O'Donnell (1999); [4] Turner et al, (1998).

Pathogenicity tests: 16 *Fusarium* isolates representative of the species *F. pseudograminearum, F. culmorum* (differing in DON/3-AcDON production)*, Microdochium* spp., *F. sporotrichioides*, *F. verticillioides* and *F. oxysporum* were tested for aggressiveness towards the susceptible durum wheat cultivar Vitron. Seeds were planted in an autoclaved potting mix (1 part sand, 1 part peat, 1 part compost) at a depth of 2 cm in each pot and were grown in a greenhouse at 25 °C. Seedlings were inoculated with an agar plug at the two- to three-leaf Zadoks's growth stage (Zadoks et al.1974). An agar plug (1 cm diameter) with mycelium was cut from the periphery of a 5-day-old culture grown on PDA (Potato Dextrose Agar). The agar plug was placed next to the stem base of each plant and covered with soil. Controls were inoculated with a mycelium free agar plug. There were three replicates per isolate with three seedlings in each replicate in a completely randomised design. All plants were watered daily with tap water and grown in a greenhouse at 25 °C and 16 h photoperiod. Twenty-one days after inoculation, each of the plants was pulled out and washed. Disease symptoms for each individual plant were assessed by calculating the proportion of the length of stem discoloration and plant height and rated using a 0–5 scale (0 = no discoloration; 1=trace to 25%; 2= 25% to 50%; 3 =more than50% to 75%; 4=more than75%; and 5=dead plant) as described by Tinline (1986). All experiments were carried out in triplicate. For each replicate, a disease index (DI) was calculated as the mean of disease scores of the seedlings. Disease severity data also were subjected to an analysis of variance. The statistical analysis of aggressiveness was based on the disease index (DI).

Results

The morphological identification of the fungal isolates (n=100) obtained from symptomatic crowns was first based on the morphological criteria reported by Leslie and Summerell (2006).

To confirm species identity and to formally distinguish some morphologically similar wheat pathogen species such *F. graminearum*; and *F. pseudograminearum*, species specific primers of mainly associated pathogen species of crown rot of wheat were used. Based on molecular identification, 3 fungal species were identified from symptomatic crowns: *F. culmorum* (25%)*, F. pseudograminearum* (21%) and *F. avenaceum* (8%) during the 3 years.

However, isolates were identified as *F. sporotrichioides* (15%), *F. verticilloides* (6%) and *F. oxysporum* (5%) based on morphological identification. For technical reasons these isolates could not be confirmed by molecular analysis. The molecular identification by DNA amplification leads to products with sizes corresponding to published values for species-specific PCR for *F. culmorum* (570 pb; Nicholson et al. 1998), F. *pseudograminearum* (523 bp, Aoki and O'Donnell 1999) which is morphologically similar to *F. graminearum* as a causal agent of FCR in Algeria and *F. avenaceum* (220 bp, Turner et al. 1998), confirming the morphological identification (Leslie and Summerell 2006).

FCR was present in all the surveyed areas. *F. culmorum*, the predominant species of FCR; was distributed in most provinces in most regions south of Algeria. Also *F. pseudograminearum* and *Microdochium* spp. were distributed in most regions in the south of Algeria.

Aggressiveness tests. All of the isolates were pathogenic, discoloration and browning of the coleoptiles were observed on all inoculated wheat seedlings, but aggressiveness among the isolates varied significantly. The most aggressive isolates belonged to *F. pseudograminearum* (ISO1, ISO4, ISO6, and ISO3) with 47.10%, 43%, 43.83% and 31.22% of the disease severity respectively. The least aggressive were *Microdochium* spp. (ISO5, ISO7), and *F. culmorum* ISO2, with 33.83%, 19.44% and 31.22% disease severity, respectively. The results of the crown inoculation showed that *F. pseudograminearum* group was the most aggressive, followed by the group *Microdochium* spp. then by the group *F. culmorum*. The analysis of variance gives highly significant results, aggressiveness among the isolates varied significantly, the F1 * F2 interaction ($p< 0.0001$). The multiple comparison of means (CMM) for (DI) using the Newman – Keuls test at 5% shows that ISO1, ISO6 and ISO4 are the most aggressive, while the ISO9 was the less aggressive.

The eight other strains of *Fusarium* spp. were pathogenic towards the variety but the aggressiveness varied greatly among the isolates in the severity of the visual disease symptoms induced (See, Disease Index (DI)). Data showed that the groups *F. verticillioides* isolates caused the disease by more than 30%, while *F. sporotrichioides* and *F. oxysporum* were the least aggressive with 20%, 5% of the disease severity respectively. The analysis of variance gave significant results, aggressiveness among the isolates varied significantly, the F1 * F2 interaction ($p< 0.004$).

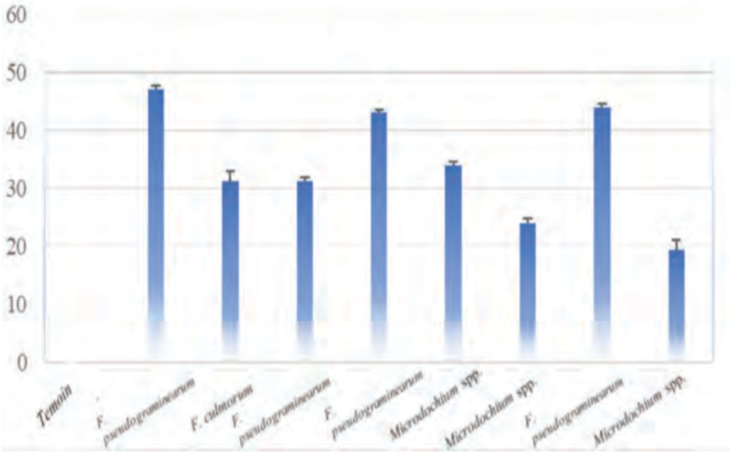

Figure. 1. Disease index (DI) of *Fusarium* spp.

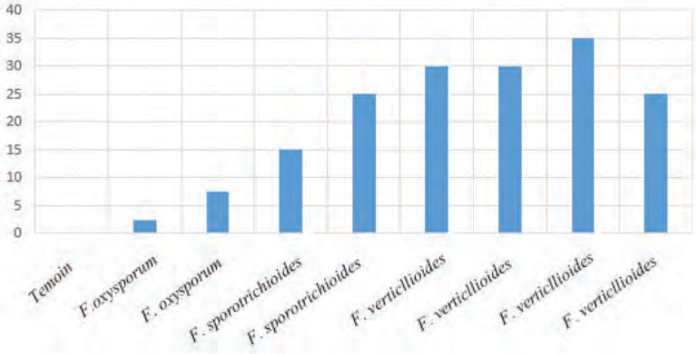

Figure. 2. Disease index (DI) of *Fusarium* spp.

Discussion

Within the last four years, there have been serious outbreaks of Fusarium crown root of wheat in Algeria. Previous studies identified *F. culmorum* as the dominant species present (Abdallah et al. 2019). This study was carried out to identify the fungal species causing FCR of wheat in south Algeria and characterize their aggressiveness. For that, diseased crowns of wheat were collected from south Algeria in 2012, 2013 and 2014. A total of 100 *Fusarium* spp. isolates originated from different regions were assayed by PCR with species specific primers. Results obtained show a diversity of species isolated from crown.

F. culmorum were the dominant species isolated from crowns of wheat in south Algeria provinces (2012, 2013 and 2014). These results are in agreement with those found by Gargouri et al. (2001). Abdellah et al. (2019) reported that in Algeria, *F. culmorum* was constantly present throughout the monitored period

(2014–2015) and was the dominant species isolated from crowns of wheat in north Algeria provinces. This result matched those obtained in previous studies conducted in other countries such as Tunisia (Rebib et al. 2014), Morocco (El Yacoubi et al. 2012), New Zealand (Bentley et al. 2006), Türkiye (Tunali et al. 2008), Italy (Scherm et al. 2013), Iraq (Matny et al. 2017), and Iran (Eslahi 2012) where *F. culmorum* was the dominant species as causal agent of FCR.

The occurrence of Fusaria is significantly affected by environmental conditions (temperature, precipitation, air humidity), and cultures practices. The species *F. culmorum* is dominant in cooler areas like north, central and western Europe (Wagacha and Muthomi 2007). The prevalence of *F. culmorum* on wheat head under Algeria climate could be explained by the occurrence of exceptional climatic conditions during flowering, a more susceptible stage to FHB infection (Touati-Hattab et al. 2016). In contrast, in dry springs and the irregular rainfall conditions, *F. culmorum* is also associated with Fusarium foot rot (Gargouri et al. 2001).

F. pseudograminearum and *Microdochium* were also distributed in most regions of south Algeria. This species has been reported in Tunisia (Kammoun et al. 2009) and Spain (Agustí-Brisach et al. 2018), it was very frequently isolated from Australian wheat (Backhouse et al. 2004, Akinsanmi et al. 2004, Hollaway and Exell 2010) and was also reported in other countries such as New Zealand (Bentley et al. 2006), Iran (Saremi et al. 2007) and China (Li et al. 2012). *M. nivale* and *M. majus* are considered as the most important causal agent of Fusarium seedling blight (Simpson et al. 2000, Waalwijk et al. 2003, Loos et al. 2004, Kammoun et al. 2009). The other species isolated from crown were *F. avenaceum, F. sporotrichioides, F. verticilloides* and *F. oxysporum* were present with a small percentage. Paulitz et al. (2002) have reported the association of these species with FCR in the Pacific Northwest of U.S.A. *F. pseudograminearum* was the most aggressive in the crown inoculation test, the results of the crown inoculation showed that *F. pseudograminearum* group was the most aggressive followed by the group *Microdochium* spp. then by the group *F. culmorum*. Dyer et al. (2009) found that *F. culmorum* caused greater seedling blight while *F. pseudograminearum* caused greater crown rot. In conclusion, in this current study we have identified species associated with FCR of wheat in Algeria. The predominant species isolated from crowns were *F. culmorum*, *F. pseudograminearum*, *Microdochium* spp. followed by *F. verticillioides*. While *F. sporotrichioides* and *F. oxysporum* were the least aggressive, the results of the pathogenicity tests show that all isolates obtained from the crowns can cause the disease on crowns.

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Occurrence of plant parasitic nematodes in cereal fields in Afyonkarahisar province of Türkiye

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Summary

Plant parasitic nematodes are significant pests causing crop loss in cereal fields in Afyonkarahisar province of Türkiye. The species plant parasitic nematodes have not been well understood in this region. Therefore, this study aimed to focus on the diversity of nematode genera and species associated with cereal crops in Afyonkarahisar during the main cereal-growing season of 2021-2022. It was evaluated to determine the occurrence of phytophagous plant parasitic nematodes and identified the cereal cyst nematode at the genus level using morphological characters. Sedentary (cyst-forming) nematodes were extracted using Fenwick's flotation and decanting techniques from 19 soil samples. Migratory nematodes were extracted using modified Baermann Funnel method from 34 soil samples. Results revealed that 14 genera of plant parasitic nematodes were detected in 95% of soil samples. *Heterodera, Pratylenchus, Pratylenchoides, Paratylenchus, Helicotylenchus, Tylenchorynchus, Trophurus, Paratrophurus, Merlinius, Amplimerlinius, Aphelenchoides, Aphelenchus, Filenchus, Ditylenchus* are the most common genera of nematodes. The cereal cyst nematodes (*Heterodera* spp.) and root lesion nematodes (*Pratylenchus* spp.) were infested with the rate of 75% and 25% of the soil samples from the surveyed fields , respectively. This study indicated that plant parasitic nematodes are widespread on cereal crops in Afyonkarahisar province more than expected. This may reveal that the plant parasitic nematodes maintain their population above the economic threshold as stimulated by the cereal monoculture system (mainly wheat) which is typical agricultural practice in Afyonkarahisar province.

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Plant parasitic nematodes in cereal fields in Uşak province, Türkiye

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Summary

Plant parasitic nematodes are significant crop pest causing damage to cereals in Uşak province of Türkiye. However, occurrence of plant parasitic nematodes has not been well understood. Therefore, this study aimed to determine the diversity of nematode genera and species associated with cereal crops in Uşak during the main cereal-growing season of 2021-2022. The incidence of phytophagous plant parasitic nematodes and identification of cereal cyst nematodes at genus level using morphological characters were studied. Sedentary (cyst-forming) nematodes were extracted using Fenwick's flotation and decanting techniques from soil samples. Migratory nematodes were extracted using modified Baermann Funnel method from 40 soil samples. *Heterodera, Pratylenchus, Pratylenchoides, Paratylenchus, Helicotylenchus, Tylenchorynchus, Merlinius, Aphelenchoides, Aphelenchus, Ditylenchus* were determined as the most common genera. The cereal cyst nematodes (*Heterodera* spp.) and root lesion nematodes (*Pratylenchus* spp.) were commonly identified in the soil samples from the surveyed fields. Results revealed that plant parasitic nematodes are prevalent on cereal crops in Uşak province. This may indicate that the plant parasitic nematodes might maintain their population above the economic threshold as stimulated by the cereal monoculture system (mainly wheat) in Uşak province.

Review of the structure of grain production and the state of cereal cyst nematodes complex in the Russian Federation

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Summary

Cereal crops are very important agricultural crops for the economy of the Russian Federation (RF). The country is one of the top five in the production and export of wheat and crude cereals. Various pathogens and pests, including root nematode complex, reduce the yield of grain crops by 10-50%, and this problem is essential for agricultural producers in the RF and in the world. The first detection of oat cyst nematode *Heterodera avenae* Wollenweber in the country was made in 1936 by V.A. Mamonov. The peak of studies on the distribution, biology, economic importance and methods of management of cereal cyst nematodes (CCN) appeared in the 1960s-1990s of the last century. The main species of cereal cyst nematodes in the RF are: *Heterodera avenae* and *H. filipjevi* (Mad.) At present, work on the study and control of populations of cereal nematodes is poorly carried out. Regular monitoring of the fields is required, as well as the selection of new nematode-resistant varieties of cereal crops.

Introduction

In the RF the cultivation of grain crops is the main direction of agrarian policy. According to the Federal State Statistics Service (Rosstat), at the end of 2020, 83 million tons of grain were harvested in Russia. According to this indicator, RF ranked third, behind China (136 million tons of grain) and India (107.6 million tons). However, the gross grain harvest in 2020 was 9.4 million tons less than in 2019.

At the same time, the total area of spring and winter wheat amounted to 29.4 million hectares, which increased by 1.3 million hectares compared to 2019. The absolute leader in wheat crops in Russia was the Rostov region - it occupies 2.87 million hectares, followed by the Altai and Stavropol Territories - 2 million hectares and 1.69 million hectares, respectively.

Unfortunately, the main areas of grain cultivation in Russia belong to the so-called “risk” farming zone, in which grain production largely depends on the prevailing weather conditions. Thus, the abnormal heat and drought in 2010 in Russia had a negative impact on crops on an area of 11 million hectares (of the total area under cereals is about 19 million hectares), as a result, the grain yield was reduced by a third compared to forecasts. Among the many factors affecting grain quality indicators, one of the most important is the rate of damage to vegetative plants by various phytopathogenic organisms (phytopathogenic fungi, bacteria, nematodes, etc.).

The most common pest of cereals is a complex of cereal cyst-forming nematodes of the family Heteroderidae (Tylenchida), including *Heterodera avenae*, *H. filipjevi*, *H. latipons*, *H. hordecalis* and other species. These nematodes are one of the most important factors of "soil fatigue" in the grain crop rotation, where cereal crops account for more than 50%. Among the hosts of these nematode species there are about 30 genera of cereals, including all types of cultivated cereals (wheat, oats, barley, rye) (Kiryanova, Krall, 1969; Popova, 1971, 1972; Tikhonova, 1972; Osipova, 1986; etc.)

History

Two economically significant species have been described in Russia: *H. avenae* (Mamonova, 1969; Popova, 1971; Tikhonova, 1972; etc.), *H. filipjevi* (Madzhidov, 1981; 1985). The species *H. latipons* is also found (Subbotin et al., 1999; and others), it is described only on wild grasses, such as *Elytrigia repens*.

In addition, five species are known to infect annual and perennial grasses. Among them are *H. arenaria* (Kiryanova, 1968), *H. bifenestra* (Kiryanova and Krall, 1969), *H. pratensis* (Gäbler, et al., 2000), *H. riparia* (Kazachenko, 1993; Subbotin et al., 2003), *H. ustinovi* (Kiryanova and Krall, 1969). At the same time, the harmfulness of these species to cultivated cereals was studied only fragmentarily.

An effective method for regulating the abundance and harmfulness of the cereal nematode complex is the use of resistant varieties of grain crops. The program for breeding wheat varieties for resistance to a group of cyst nematodes was carried out during the former USSR in 1970-1980, but was closed due to a decrease funding for science in the period 1980-1990.

Status

The structure of grain production in recent years are presented in Table 1. An increase in sown areas and wheat yields was noted. However, the control of the nematode fauna of this important crop remains neglected.

Table 1. The structure of grain production in Russia in 2015-2020

Indicator/years	**2015/16**	**2016/17**	**2017/18**	**2018/19**	**2019/20**
Wheat production (thousand tons)	61044	72529	85167	71685	74500
Export of grain (thousand tons)	8465	9258	11379	7402	11465
Sown areas under wheat (thousand ha)	27709	27924	27264	28092	29421
Wheat yield (centner/ha)	26.8	31.2	27.2	27	30.3

In the European part of Russia, only *H. filipjevi*, *H. pratensis*, and *H. latipons* are widely represented. The dominant species in the main grain-growing regions of Russia is Filipyev's cyst nematode *H. filipjevi*, which infects all types of cereal crops to varying degrees.

As for the species of oat cyst nematode *H. avenae*, according to the results of studies conducted in 1960-1990, this species was dominant throughout the territory of the former USSR, but, according to some authors, this species is actually Filipyev's cereal cyst nematode *H. filipjevi*, correctly described only in 1981-1985 by A.P.

Majidov (Majidov, 1981, 1985; Subbotin, Mundo-Ocampo, Baldwin, 2010). At present, it is impossible to confirm or refute this hypothesis, due to the lack of an acceptable amount of up-to-date data on the distribution of these types of cereal nematodes in Russia. In addition, there is a possibility that the populations of cereal nematodes found earlier in the Volga, Ural, and East Siberian regions may be mixed. For example, in the USA (Oregon), *H. filipjevi* occurs in mixed populations with *H. avenae* (Smiley et al., 2008; Smiley, 2009).

In the "State Register of Breeding Achievements Approved for Use" there is a sufficient range of nematode-resistant varieties of both domestic and foreign selection. However, the majority of cultivated wheat varieties (95.4%) are descendants of such most successful varieties as Bezostaya 1 (67%) and/or Mironovskaya 808 (31%), i.e. cultivar resistance genes are not diverse. One of the promising directions in breeding for resistance to cereal nematodes is the use of wild relatives of modern wheat as resistance gene donors.

Impacts

The wide distribution and high level of harmfulness of cereal cyst nematodes of the genus *Heterodera* are found in agronomic zones where the saturation with grains in the crop rotation is more than 60-70% (Popova, 1975). Grain yield losses from a complex of cereal nematodes in the agronomic and climatic conditions of Russia can reach 8–11 centners/ha (up to 70%) for wheat and 3–4 centners/ha (up to 25–30%) for barley (Mamonova, 1969). Losses of wheat in the Novosibirsk region reached 4-8 centners/ha (Zhuk, 1967; Shiabova, 1969). The yield of wheat infected with *H. avenae* in the Tyumen region was 2 times lower than usual (Safyanov and Skripnik, 1968). Rye and corn are more resistant to the oat nematode (Tikhonova, 1968).

Thus, the share of cultivation of the main grains (wheat, oats, barley) in comparison with other crops of crop rotation (rowed, industrial and fodder) has increased in the country in general by more than 10% (from 47.4 to 58.40%) from 1992 to 2010. As mentioned above, the wide distribution and high level of harmfulness of cereal cyst nematodes of the genus *Heterodera* are found in agronomic zones where the grain saturation in the crop rotation is more than 60-70%.

At present, in the Volga region, the share of grain according to Rosstat for 2010 ranged from 45.94% (Saratov region) to 63.57% (Samara region). In the Urals region, this figure is also approaching a critical level (from 55.96 to 79.10%). Suchwise in the Chelyabinsk region, the share of grain in 2010 was 68.86% (in some types of farms up to 93%)

Prospects

The Russian Federation has increased grain production in recent years. Most of this volume is wheat grain. Russia has significant areas of agricultural land. The possibilities of increasing the sown area in each of the regions of the country (including chernozem soils) are far from being fully realized. Also, to increase the volume of grain production, a more rational selection of varieties and technologies can be used in accordance with the climatic characteristics of the regions.

To reduce crop losses associated with cereal nematodes, monitoring of these pathogens is essential. And taking timely measures when the site of infection is detected. The main method of regulating the abundance and harmfulness of the complex of cereal nematodes, if it is impossible to reduce the share of cereal crops in the crop rotation, is the use of resistant varieties and hybrids of wheat, oats and barley.

Recommendations

Agrotechnical methods are remaining the dominant methods for regulating the number and harmfulness of cereal nematodes, in conditions of the absence of registered nematicide both in the Russian and European markets.

Among them: the use of multi-field crop rotations to reduce the share of cereals in the total volume of cultivated crops (the least profitable method in modern economic conditions); and the use of crop varieties that are resistant to these types of pathogens. Successful implementation of this approach requires regular monitoring of cereal nematodes, as well as the selection of new varieties with the involvement of new resistance genes.

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Current state and prospects of national development of agricultural research and innovations in Azerbaijan

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Summary

After gaining independence, as well as a result of the receipt of oil revenues in Azerbaijan, the state began to pay great attention to the development of agriculture and agricultural research. Since agrarian and land reforms were carried out in the republic, the relevant state agricultural structures were optimized, policy decisions were made, and relations with foreign countries and international organizations in the field of agriculture expanded. However, currently there are some problems in agricultural research in Azerbaijan, about which the article describes in detail and suggests ways to solve them.

Introduction

The Republic of Azerbaijan is located in the South Caucasus, on the western coast of the Caspian Sea, surrounded by the mountains of the Greater and Lesser Caucasus and the Talysh Mountains, and it borders on the north with the Russian Federation, on the south with Iran, on the west with Türkiye and Armenia. The population is over 10.0 million people. Territory: 86.6 thousand km^2: 58% of the territory is occupied by mountains, 42.0% by plains; 27.0% of them are above 1000 m, 18% are 26 m below the level of the world ocean. Azerbaijan has unique natural and climatic conditions that provide opportunities for the formation of diversified and productive agriculture. The lowland and foothill steppes of the country are suitable for the development of irrigated agriculture, and mountainous areas are suitable for rain–fed agriculture and animal husbandry.

Agriculture is a traditional sphere of production in Azerbaijan. The basis of agriculture is occupied by two large branches - crop production and animal husbandry. Grain growing, cotton growing, vegetable growing, potato growing, fruit growing, viticulture and tobacco growing predominate in crop production, while cattle breeding, sheep breeding and poultry farming predominate in animal husbandry.

According to data as of January 1, 2022, 55.0% of the country's territory is land suitable for agriculture (of which 16.5% is irrigated). 37.8% of these lands are plots suitable for sowing, 4.7% are occupied by perennial plants (orchards, vineyards, etc.), 36.6% are hayfields and pastures, 12.0% are forests. The remaining plots belong to the lands of the state reserve, settlements, nature reserves, etc. 0.56 hectares of land suitable for agriculture and 0.21 hectares of acreage are due per capita. In 2022, out of 1032.0 thousand hectares of the total sown area of the republic, grain crops were sown on 985.7 thousand hectares: winter wheat, winter barley, spring grain crops – legumes, cucumbers, rice, spring wheat, etc., and on the remaining 46.3 thousand. other agricultural crops. The rich natural environment of Azerbaijan (abundance of sunny days, biological activity, the presence of most climatic zones (the presence of 9 zones out of 11), etc.) makes it possible to harvest in several districts 2-3 times a year.

History

Reforms in the agricultural sector. After 1993, the Government of Azerbaijan began to take measures in connection with the privatization of property, land and the distribution of land between the population living in the village and working in this industry. In subsequent years, prices were liberalized, the privatization process was extended and restrictions in export operations and in the market of material and technical resources and food were eliminated. As a result of consistent and targeted comprehensive measures implemented in the country after the second half of 1993. a decrease in production in the agricultural sector was prevented, and a strong and reliable foundation was laid for the development of many important sectors of the economy through market relations. And also, since 1996, there has been an increase in microeconomic indicators of agriculture.

At the end of 1996, collective farms and state farms were completely liquidated, their land plots and property were distributed among the peasants. Since 1997, state and collective agricultural enterprises have been privatized in an accelerated manner, new private economic forms, farms and other entrepreneurial farms have been created. The "State Program of socio-economic development of the regions" covering 2004-2008 was approved. The program establishes priority areas to accelerate reforms in the agricultural sector and provides for the implementation of the following measures:

- Development of agricultural products processing enterprises.
- Direction for the restoration of public investments, in particular industrial enterprises, improvement of regional infrastructure and provision of public services and the construction of social facilities.
- Application of tax exemption for agricultural producers.
- Continuation of the implementation of state financial support programs for enterprises of agricultural and industrial complexes, farms, preparation of a scheme for issuing micro-loans to various segments of the population engaged in entrepreneurial activities and the creation of credit unions, micro-credit banks that will finance projects in this sector, stimulation of organizations of the insurance system in agriculture based on market principles.
- Provision of comprehensive state support for the sale, both on the domestic and foreign markets, of products grown by farmers, the creation of an Export Support Fund, exchanges, wholesale warehouses, auctions.
- State support and financing of veterinary and sanitary, phytosanitary works, technical control, seed production and livestock breeding.
- Support and creation of a network of agricultural services.
- Restoration and reorganization of existing land reclamation and irrigation systems, improvement of water supply of irrigated lands through the construction of new systems, prevention of soil salinity, unhindered flow of main channels and other waters into the Caspian Sea.
- Elimination of water shortage, construction of new reservoirs, main canals and other water management facilities for this purpose.
- Implementation of certain measures against soil erosion.

- Preparation of soil reclamation projects.
- Preparation of the land cadastre.
- Creation of a land market in the country.
- Simplification of procedures for registration of ownership rights, use and lease of land plots.

State support for agricultural producers:

1. According to the decree of the President of the Republic of Azerbaijan dated January 23, 2007, producers of agricultural products are allocated 50 USD per hectare from the state budget for the use of fuel and motor oils on sown areas;
2. In addition, an additional USD 50 is allocated for sowing wheat and rice for each hectare of sown area.
3. Mineral fertilizers are sold to agricultural producers with a 50% discount;
4. For the products sold to seed farms and nurseries, additional funds are allocated from the state budget for seeds and seedlings of the first reproduction 40% above the realized price, for seeds and seedlings of the second reproduction -30%;
5. For the production of original, super elite and elite seeds, farms are allocated 100% of the funds spent;
6. At the expense of the state budget only in 2005, 9467 pieces of agricultural machinery were purchased, including 990 combine harvesters, 2494 tractors, 140 excavators, 5483 agricultural machinery and equipment for various purposes. All of them are leased for a period of 10 years to legal entities and individuals with partial prepayment of the cost of machinery and equipment (initial payment of 20%);
7. In order to improve the pedigree composition, 770 heads of breeding heifers were purchased, which were sold to livestock producers by leasing on preferential terms;
8. The purchase of breeding cattle and their sale to local entrepreneurs at a discount of up to 50% will continue.

Static studies of the last 25 years show that as a result of the above measures taken in the structure of crops, the share of cereals, legumes, potatoes, vegetables and melons has sharply increased, and the share of grapes, cotton and tea has decreased.

As a result of the consistent deepening of agrarian reforms, the directions of production in agriculture, the volume of production gradually changed, and the formation of a crop assortment based on competition began. In such conditions, the economic efficiency of agricultural production technologies and their compliance with modern requirements are becoming important for farmers.

Situation

In order to increase the role of science in the agrarian reforms carried out in the republic, eliminate the lag, parallelism and disunity in this area, to form a unified strategy in scientific works, the Azerbaijani Agrarian Scientific Center (now the Agrarian Innovation Center) under the Ministry of Agriculture was established by Resolution of the Cabinet of Ministers of the Republic No. 190 of December 16, 1999. The structure of the Agricultural Innovation Center now includes 7 research institutes, 8 regional agricultural research centers and 40 experimental production bases. In addition, there are 6 research institutes of agricultural profile in the republic as part of the National Academy of Sciences, 2 institutes as part of the Open Joint Stock

Company of Land Reclamation and Water Management and 2 institutes as part of the Ministry of Ecology and Natural Resources of the Republic of Azerbaijan.

The main purpose of agricultural science is to conduct scientific research in the regions of the republic in priority areas of agriculture, the formation of thematic plans in the interests and at the request of farmers and peasants, the introduction of research results in farms and the organization of a system for the dissemination of these results, strengthening the human potential and material base of science. To achieve this, it is necessary to improve the planning and financing of scientific research. Here the key role is played by the choice of topics that meet the requirements of farmers and peasants. The obtained scientific results should form the "purchase and sale" market.

In order to consistently address the issues posed to the agricultural research system and further deepen reforms in the field of science, the activities of the Agrarian Innovation Center were aimed at improving agricultural research, determining their priorities, training personnel, forming regional scientific structures for the dissemination and implementation of scientific results, etc. The Agrarian Innovation Center has carried out some work to establish scientific cooperation between research institutes that are part of the Ministry of Agriculture and with other research institutions of the Republic of agriculture. First of all, the coordination of research topics was carried out in order to avoid parallelism.

As a result of the integration of Azerbaijan's agricultural science into international scientific organizations, there are certain innovations in improving research methods. An example of this is the collection of genetic resources of plants and animals that meet international standards, the creation of joint varieties, etc. In connection with the organization of agricultural production in accordance with market economic relations and the need to solve important agricultural problems on a scientific basis, the Regional Agricultural Innovation Centers are tasked with: the introduction and dissemination of the results of research work in different natural and economic zones by laying demonstration experiments of new varieties of crops; holding exhibitions of new breeds of animals, new agricultural machinery; selection of new high-yielding varieties of agricultural crops, breeds of farm animals, planting materials that most correspond to the soil and climatic conditions of the zone, determination of the need for them; identification of issues that farmers urgently need to solve.

Relations with international organizations and foreign countries

Since 1994, more than 20 projects have been implemented in the country's agriculture by various international organizations and states: 7 TACIS programs (1994-2000), 2 World Bank (1997-2001), 3 Food and Agriculture Organization of the United Nations (1999-2001), 1 International Fund for Agricultural Development, 1 Organization of the Black Sea Economic Cooperation and 2 of the European Commission (1997-2002), 1 of the Islamic Development Bank, 3 of Germany, 2 of the Netherlands, 1 of Japan.

On the basis of the TACIS program for planning and reorganization of agriculture, projects were implemented to strengthen the capabilities of the Ministry of Agriculture, create private farming groups, assist them in the production of food products for self-sufficiency and marketing, support the private sector in the food industry in the regions, establish political means that give impetus to agrarian reform, reorganize trade agriculture, ensuring food security, supporting the private agricultural sector, creating a grain exchange, assistance in creating credit unity in villages, assistance to commercial and professional farms in Azerbaijan, etc.

The Food and Agriculture Organization of the United Nations in Azerbaijan financed the processing of the medium-term policy of the development of the agricultural sector, the improvement of legislation on phytosanitary, inventory of land use and vegetation cover by funding from a long distance. The International Fund for the Development of Agriculture (IFAD) and the International Bank (WB) in Azerbaijan have implemented projects to organize the provision of advisory and information services in relation to all branches of agriculture to farmers operating in the territories of former collective farms and state farms, the privatization of land and property, the formation of new entrepreneurs in villages, the preparation of a mechanism for their credit, preparation of political and economic strategies that serve to increase incomes and yields in agriculture.

On the basis of the protocol concluded between the European Commission and the Government of Azerbaijan, funds were allocated to improve the extra-budgetary financial structures of the agro-industrial complex, loans were issued to producers of agricultural products.

The Islamic Development Bank has allocated a loan for the construction of the Milo-Mugan collector.

Cooperation was carried out by the International Center for the Improvement of Corn and Wheat (CIMMYT) in the field of grain crop breeding and training, the International Center for Agricultural Research in Arid Regions (ICARDA) in the field of genetic resources, seed production, Breeding and Training, the International Institute of Plant Genetic Resources (IPQRI) on the use, conservation and Accumulation of Genetic Resources, the International Research Institute of Agricultural Crops in Semi-Arid Tropical Zones (ICRISAT), related to the exchange and selection of the gene pool of oil and legumes, the International Service for National Agricultural Research (ISNAR) in the field of agrarian reform. There is a joint Azerbaijani-Turkish enterprise for seed research, established jointly with the Turki Ishbirlii ve Kalkynma Ajansy (TIKA). In the Guba Regional Center of Agrarian Science, work is underway to enrich the gene pool of fruit crops and 2000 pieces were brought and planted from France as part of a three-year project. virus-free oculation of apple trees (four varieties of apple trees, 10 pcs. each).

According to the memorandum on financial and technical cooperation between the Federal Republic of Germany and the Government of the Republic of Azerbaijan, the projects “Agrarian Policy” and “Support for regional cooperation and stability, food security in the South Caucasus”, the project “Agriculture of Ganja”, financial support of the Embassy of the Kingdom of Norway in the Republic of Azerbaijan were implemented. The Governments of the USA, Great Britain, Türkiye, Switzerland, Denmark, Belgium, Japan, Austria, Holland, Israel, Italy, Brazil, Germany, China, France, Greece, Saudi Arabia, Pakistan, Iran, India, Egypt, Bulgaria, Russia, Belarus, Kazakhstan, Kyrgyzstan, Uzbekistan, Ukraine, Estonia and other countries have signed agreements providing for cooperation in the agricultural sector. The Agricultural Innovation Center and the Research Institute of this system cooperate with many international organizations (ICARDA, CIMMYT, CIP, ILRI, IWMI, FAO, ECO, TIKA, Bioversity International, etc.) on research, collection and storage of genetic resources, the creation of new varieties of crops and their environmental testing, also in the field of research in animal husbandry, the introduction and dissemination of advanced technologies in agricultural production, as well as on other problems of agricultural research. Since 1995, the Azerbaijan Research Institute of Agriculture has been successfully carrying out international scientific and technical relations with ICARDA and CIMMYT on grain crop breeding, genetic resources, seed production and personnel training. According to the results of the joint work of ICARDA, CIMMYT and the Research Institute of Agriculture, varieties of soft wheat – "Azamatli 95", "Nurlu 99" and "Gobustan", barley – "Baharly", chickpeas – "Narmin" and lentils – "Arzu" were zoned. Currently, these varieties are sown in Azerbaijan on an area of more than 250 thousand hectares

Problems in the agricultural sector:

- In accordance with international requirements, incomplete formation of a quality control mechanism, product packaging, certification and standardization reduces the competitiveness of products and restricts exports.
- Outdated irrigation and drainage systems.
- Exposure to erosion and salinization of agricultural land.
- Weak development of the system of mechanization and automation of agricultural production.
- Reduced profitability and turnover of small farms, weak opportunities for the acquisition and implementation of the required technologies by them.
- Problems in the field of personnel training and weak material and technical base of research institutes;
- Modernization and expansion of rural infrastructure.
- Insufficient formation of a credit system and banks in rural areas, restriction of farmers' access to financial sources.

The need for investment:

- Creation of enterprises for the production of veterinary drugs.
- Establishment of enterprises in rural areas for the storage and packaging of exported products.
- Processing of cotton and silk.
- Storage and processing of early potatoes (mashed potatoes, chips, flour, starch).
- Development of fruit, vegetable and grape processing (wine, juices, kishmish, vinegar).
- Production and processing of environmentally friendly agricultural products.
- Modernization of greenhouses.
- Creation of enterprises for the production of small-sized agricultural machinery and equipment.
- Cooperation support.
- Support for agromarketing.
- Modernization of research institutes in priority sectors.
- Training of personnel in the field of standardization, certification, quality control and agromarketing.

The Prospects

In order to increase the necessary legal, economic and professional knowledge of farmers in research institutes, regional agricultural research centers, as well as in farms, seminars, meetings and training courses are held periodically. More than 100 new varieties of agricultural plants and a number of production technologies have been demonstrated on the experimental fields of farmers, seminars with the participation of farmers are held periodically.

For the development of agricultural science in the following areas, we consider priorities in the strategy of agricultural science:

1. Rational use of land and water resources (groundwater management, prevention of erosion and salinization, increasing soil fertility).
2. Protection of biodiversity, collection and use of genetic resources of plants and animals.
3. Breeding, seed production and nursery of agricultural crops.
4. Diversification of the crop production system with the inclusion of leguminous oilseeds and industrial crops.
5. Development of a mountain agricultural system.
6. Integrated plant protection system.
7. Improvement of breed composition and breeding works (especially dairy cattle breeding and dairy sheep and poultry farming), preservation of animal health.
8. Forage production, management of the use of natural pastures.
9. Storage, processing and marketing of agricultural products.
10. Socio-economic research on the problems of agricultural sector development.
11. Improving the professional level of scientists, especially young scientists and agricultural specialists, by establishing links with international scientific centers.
12. Updating the national system of agricultural research.
13. Taking into account zonal principles, clarify priority research in the system of agriculture (crop rotation, seed production, tillage, plant protection, fertilizers, irrigation, etc.).

Priority crops are:

- Cereals (wheat, barley, corn).
- Legumes (chickpeas, lentils, beans).
- Technical (cotton, sugar beet).
- Fodder (alfalfa, esparcet).
- Oilseeds (sunflower, soy, etc.).
- Potatoes.
- Vegetables (tomato, cucumber, onion, watermelon, melon).
- Fruit (apple, pear, pomegranate).
- Grapes.

To manage and mitigate the effects of drought, the following measures are provided:

- Identification of the most vulnerable areas of farmland in relation to drought.
- Study of erosion processes and determination of the exact volume of territories subject to erosion to varying degrees.
- Study of the water reserve of the republic, determining the location for the creation of small reservoirs in foothill and mountainous areas in the most arid areas, the introduction of water-saving irrigation technologies.
- Creation of new plant varieties that are drought-resistant and resistant to adverse conditions.

- Development and application of new agrotechnical techniques that preserve soil moisture, reject erosive processes, such as minimal processing, ridge sowing, linking and terracing on sloping lands, etc.
- Carrying out an event to improve pastures and haymaking to increase the productivity of small farmers engaged in sheep farming.

The modernization of agricultural research is to provide agricultural producers, as well as consulting and marketing workers, with the necessary technology, training and consistent supply of important information to them. The modernization of the Azerbaijan Agricultural Research Institute has been completed and after that we believe that the institute will work more closely with farms producing agricultural products and processing enterprises. .The results of the implementation of reforms in the field of agricultural science according to the “State Program for the implementation of the National Strategy in the Republic of Azerbaijan in 2009-2015” will contribute to a significant improvement in the scientific support of agricultural production, which will give farmers the opportunity to produce competitive products.

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