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Identification and Evaluation of Propagation Techniques of Dianthus orientalis Adams.

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ABSTRACT: *The aim of the present study was identification and evaluation of propagation techniques of* ***Dianthus orientalis*** *Adams., a Turkish native plant which has good potentials as a landscaping plant with conspicuous pink flowers in autumn. Three different propagation methods were used in this study. These methods are the propagation of* ***D. orientalis*** *by seed, cuttings and* ***in vitro****. The seeds and cuttings of this species collected from wild were used as the experimental material. The germination rate of the seeds and the rooting rate of the cuttings were determined. An effective surface sterilization protocol for in vitro seed germination of* ***D. orientalis*** *was developed. The seeds were germinated on MS medium. Germination success of the seeds on MS medium was determined.*

Key words: Dianthus orientalis*, cutting, germination, rooting, seed,* ***in vitro.***

Dianthus orientalis Adams.'ın Üretim Tekniklerinin Belirlenmesi ve Değerlendirilmesi

ÖZ: *Bu çalışmanın amacı, Türkiye doğasında bulunan ve sonbaharda son derece dikkat çekici pembe çiçekleriyle peyzaj bitkisi olarak değerlendirilme potansiyeline sahip olan* ***Dianthus orientalis*** *Adams.(Yar karanfili)'ın üretim tekniklerinin belirlenmesi ve değerlendirilmesidir. Bu çalışmada üç farklı üretim yöntemi kullanılmıştır. Bu yöntemler* ***D. orientalis****'in tohumla, çelikle ve* ***in vitro*** *ortamda üretimidir. Deneme materyali olarak, bu türün doğadan toplanan tohumları ve çelikleri kullanılmıştır. Tohumların çimlenme oranı ve çeliklerin köklenme oranı belirlenmiştir****. D. orientalis****'in in vitro tohum çimlenmesi için etkili bir yüzey sterilizasyon protokolü geliştirilmiştir. Tohumlar MS ortamında çimlendirilmiştir. Tohumların MS ortamında çimlenme başarısı belirlenmiştir.*

Anahtar kelimeler: Dianthus orientalis*, yar karanfili, çelik, çimlenme, tohum,* ***in vitro.***

INTRODUCTION

The genus *Dianthus* L. belongs to Caryophyllaceae family and includes approximately 300 species distributed in Europe, Asia, Africa and North America (Reeve, 1967). The mainly center of genus diversity is the Mediterranean area (Bittrich, 1993). They are commonly known as pinks and carnations. The plants are annual, biennial or perennial. *Dianthus orientalis* Adams. is a perennial herbaceous species which has a wide distribution through South and East of Turkey. It is mostly seen in calcareous slopes, rocky areas, cliffs and lime-free rocks which have structure of the schist or gneiss and at altitudes ranging from 20 m to 200 m. It is a very attractive with pink flowers throughout autumn on 25-70 cm stems. Flowers are usually solitary, sometimes on the branches. It has a good potential of being landscape plant. This species is particularly blooming in the autumn when there are few flowers. It can also be a good erosion preventing plant on slopes, since it has extensive creeping woody base. Any scientific

research on propagation of *D. orientalis* was not found in literatures. Methods of propagation of this species should be known both in terms of conservation and utilization of its potential as an ornamental plant. Many *Dianthus* species can be propagated by seeds, cuttings and tissue culture.

Dianthus species such as *D. barbatus*, *D. chinensis*, *D. plumarius* and *D. gratinanopolitanus* were easily produced with both seed and cutting (Galbally and Galbally, 1997; Hartmann *et al.*, 2002).

Pre-treatments such as submersion in hot water, mechanical or chemical scarifications and hot air are used to break dormancy related to seed coat while the cold and warm stratifications are usually applied to break the dormancy caused by some restrictions at the embryo level (Landis *et al.*, 1996). Some enzymes and growth regulators, such as auxins, cytokinins and gibberellins have critical role on breaking dormancy and germination of seeds. Among these growth regulators, gibberellic acid has important influence on them (Riley, 1987). Exogenous GA stimulates amylase activity. Aleurone layer of endosperm is sensitive to GA (Fincher, 1989). GA also causes release of enzymes amylase and protease. These enzymes participate in the breakdown of stored starch to simple sugars (Gubler *et al.*, 1995). These sugars are then translocated to growing embryo where they provide energy for growth (Mayer and Poljakoff-Mayber, 1989).

Auxin-type growth regulators have been used to increase the percentage of cuttings that form roots, root initiation, increase the number and quality of roots per cutting and increase uniformity of rooting (Hartmann *et al*., 1990). Indole butyric acid (IBA) has been the most common rooting hormone for general use because it is generally not phytotoxic over a wide concentration range and is effective in promoting rooting a large number of plant species (Hartmann *et al.*, 1990). Exogenous auxin application improves rooting efficiency and quality of stem cuttings, while IBA and NAA stimulate adventitious rooting in cuttings (Copes and Mandel, 2000).

There is no previous report on propagation of *D. orientalis*.

The objective of this study was to determine rooting rate of their cuttings, germination success in greenhouse and *in vitro* propagation of *Dianthus orientalis* seeds, and to identify suitable propagation method for *D. orientalis.*

MATERIALS AND METHODS

Plant material: The cuttings and mature seeds of *D. orientalis* were collected from Kemer-Çamyuva-Turunçova locations (36^{o} 28’ 02’’E and 40^{o} 51’ 91’’N - 36^{o} 28’ 12’’E and 40^{o} 51’ 44’’N - 36^{o} 24’ 36’’E and 40^{o} 32’ 25’’N; 22-20-85 m, respectively) in Antalya Province. The seeds were removed from capsules and cleaned. Seeds with the same size, shape and color were selected for use in the experiments. In addition, their maturity was the same. In this study, the herbaceous cuttings of 7-10 cm length taken from the shoot tips of *D. orientalis* were used. The cuttings were transferred from the natural habitats in the ice box. Cuttings were taken on April.

Propagation techniques

Three propagation methods of *D. orientalis* were used in this study.

1. Rooting of cuttings: In this study, the herbaceous cuttings of 7-10 cm length taken from the shoot tips of *D. orientalis* were used. The cuttings were transferred from the natural habitats in the ice box. The basal ends of the cuttings were dipped into IBA solutions at 50, 100, 500, 1000, 2000 and 3000 mg/l for 10 seconds. Only the distilled water treatment was applied to the control group cuttings. After treatment, *D. orientalis* cuttings were planted into three different rooting media that contained peat, perlite and their mixture (1:1 / v:v). The study was carried out in a nursery belonging to a private company in Antalya. After 26 days from planting, the cuttings were removed from the rooting medium and rooting rates were determined. Each treatment had 3 replicates containing 20 cuttings.

2. Greenhouse seed germination: To determine the effect of pre-sowing treatment on seed germination, three different treatments were applied.

The treatments were;

i) Dipping in 10, 50, 100, 250, 500, 1000 and 2000 mg/l giberellic acid (GA_3) solutions for 24 hours,

ii) Soaking in hot water at 50, 60 and 70^{o}C for 2 minutes,

iii) Store at 5 and 10^{o}C for 20 and 40 days in the storage room. Control seeds were sown without any treatment.

The study was conducted in a commercial nursery in Antalya. The seeds were sown soon after fungicide (Thiram) treatment in styrofoam trays containing peat, perlite and their mixture (1:1 / v:v). A thin vermiculite layer was spread over cells of the trays to keep the moisture. The trays were irrigated and wrapped with plastic folia, before they were placed in a germination room which had 22 ^{o}C temperature, 60-70% relative humidity and darkness. In the third day, seeds started to germinate and then the trays were transferred to the nursery. Throughout the study, minimum temperatures were kept at 9 ^{o}C, maximum temperatures were at 33 ^{o}C and average relative humidity was 44 % in the greenhouse. Germination rates were recorded daily. Each treatment had 10 seeds in 3 replications.

3. *In vitro* seed germination: Pre-sowing treatments: In this study, three different pre-sowing treatments were applied in order to enhance *in vitro* seed germination:

i) Soaking in 0, 10, 50,100, 250 mg/l of gibberellic acid (GA_3) solutions for 24 hour,
ii) Soaking in hot water at 50, 60, 70^{o}C for 2 minutes and control,
iii) Storing at 5 and 10^{o}C for 30, 45, 60 days in a controlled storage room and control.

Sterilization: At first, pre-treated seeds were washed with tap water for 6 hours. Then, they were surface sterilized in 0.15% Benomyl (fungicide) including two drops of Tween 20 for 10 min and then rinsed three times in sterile distilled water. Finally, it was followed by surface sterilization with 10% sodium hypochlorite solution for 5 min. and again rinsed three times in sterile distilled water.

***In vitro* culture media and conditions:** The seeds were placed into glass jars, contained with 40 ml modified MS (Murashige and Skoog, 1962) basal nutrient medium. The cultures were maintained at 25^{o}C under a 16/8 h (light/dark) photoperiod with 3000 lux light irradiance provided by cool-white fluorescent tube. Germination rates were recorded daily. Each treatment had 3 replicates containing 10 seeds.

Statistical analysis: All the experiments were set up in a completely randomized design. Germination studies both in greenhouse and *in vitro* conditions had 3 replicates containing 10 seeds. Rooting study had 3 replicates containing 20 cuttings. Data were statistically analyzed using SAS (Anonymous, 1985). Means were compared by Duncan's multiple range tests at 5% probability (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Rooting of Cuttings

In this study, the effects of media ($P<0,001$), IBA concentrations ($P<0.001$) and media x IBA concentrations interactions ($P<0.01$) on rooting were found to be significant (Table 1).

The best result was obtained from cuttings which were treated with 2000 mg/l IBA and planted in peat medium (Figure 1). The rooting rate of these cuttings was 28%. Control cuttings gave the lowest rooting rate with 2%. The rooting of cuttings was found higher in peat medium than the other ones. In herbaceous cuttings, IBA treatments of 1000-2000 ppm usually yielded successful results (Johnson *et al.*, 2006). Pilon (2006) reported that *D. gratianopolitanus* 'Firewitch' cuttings were rooted in 3-4 weeks when they were immersed in 750-1000 ppm IBA solution and planted in the rooting medium. In rooting studies, perlite was the most unsuccessful medium in terms of rooting rate and seedling development.

Table 1. Effects of different IBA concentrations and growing medium on rooting of *D. orientalis* cuttings in the nursery conditions.
Çizelge 1. Anaçlık koşullarında *D. orientalis* çeliklerinin köklenmesi üzerine farklı IBA konsantrasyonları ve yetiştirme ortamlarının etkileri.

IBA (mg/l)	Rooting rate / Çelik köklenme oranı (%)			Mean Ortalama
	Growing medium			
	Peat	P+P	Perlite	
0	2 c[1]	5 b	0 b	2 C[x]
50	7 c	2 b	5 ab	5 C
100	3 c	8 b	3 ab	5 C
500	7 c	15 ab	2 ab	8 BC
1000	25 ab	10 b	3 ab	13 AB
2000	28 a	13 ab	7 ab	16 A
3000	13 bc	25 a	8 a	15A
Mean	12 A	11 A	4 B	

F values: Media: ****, IBA: ****, Media x IBA: ***.
Means in the same columns and lines followed by the different letters are significantly different according to Duncan's Multiple Range Test (p≤0.05).
, *, **** significant at 0.05, 0.01, 0,001 respectively. CV (%): 72,39.
F değerleri: Ortam: ***, IBA:****, Ortam x IBA***
Aynı satır veya sütunda farklı harfle gösterilen ortalamalar Duncan Testine gore istatistiksel olarak (p≤0,05) farklıdır.
, *, **** istatistiksel olarak sırasıyla 0,05, 0,01 ve 0,001 düzeyinde önemli. VK (%): 72,39.

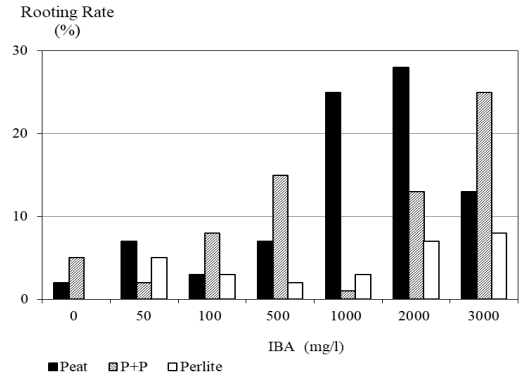

Figure 1. Effects on rooting of *D. orientalis* cuttings of the interactions between IBA and medium.
Şekil 1. IBA ve ortamlar arasındaki interaksiyonların *D. orientalis* çeliklerinin köklenmesi üzerine etkileri.

Greenhouse Seed Germination

The difference between GA_3 concentrations was found to be significant (P<0.05). On the other hand, the effects of media and media x GA_3 interaction on germination were not significant (Table 2).

2000 mg/l GA_3 applied seeds had the highest germination rate (30%) compared to other GA_3 applications and control. Germination rate was increased with increasing concentrations of GA_3, except for 250 mg/l GA_3 concentration, in the peat medium. The seeds which were applied with 2000 mg/l giberellic acid and sown in peat medium gave the best result with 40% germination rate (Figure 2). Similarly, pre-sowing treatment with GA_3 stimulated seed germination of Cleopatra mandarin and Rangpur lime rootstocks in comparison to control and the most successful treatment was 2000 ppm (Sharaf *et al.*, 2016).

Table 2. Effects of different GA_3 pre-sowing treatments and growing medium on germination of *D. orientalis* seeds in the greenhouse conditions.
Çizelge 2. Sera koşullarında *D. orientalis* tohumlarının çimlenmesi üzerine GA_3 ön uygulamaları ve yetiştirme ortamlarının etkileri.

GA_3 (mg/l)	Germination / Çimlenme (%)			Mean
	Growing medium			
	Peat	P+P	Perlite	
0	0.10 b[1]	0.23 a	0.20 a	0.18 AB[x]
10	0.10 b	0.20 ab	0.20 a	0.17 B
50	0.17 b	0.10 b	0.30 a	0.19 AB
100	0.17 b	0.17 ab	0.10 a	0.15 B
250	0.07 b	0.17 ab	0.13 a	0.12 B
500	0.17 b	0.03 b	0.17 a	0.12 B
1000	0.17 b	0.13 ab	0.23 a	0.18 AB
2000	0.40 a	0.37 a	0.13 a	0.30 A
Mean	0.17 A	0.17 A	0.18 A	

F values : GA_3:**, Media: NS, Media x GA_3: NS.
Means in the same columns and lines followed by the different letters are significantly different according to Duncan's Multiple Range Test (p≤0.05).
**, NS: Significant at 0.05 and non-significant, respectively. CV (%): 70.95
F değerleri: GA_3: **, Ortam: ÖD, Ortam x GA_3: ÖD
Aynı satır veya sütunda farklı harfle gösterilen ortalamalar Duncan Testine gore istatistiksel olarak (p≤0,05) farklıdır.
**, ÖD: Sırasıyla istatistiksel olarak 0,05 düzeyinde önemli ve önemli değil. VK (%): 70,95

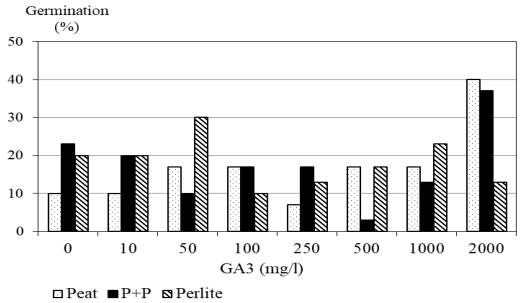

Figure 2. Effects on germination of *D. orientalis* seeds of the interactions between GA_3 and medium.
Şekil 2. GA_3 ve ortamlar arasındaki interaksiyonların *D. orientalis* tohumlarının çimlenmesi üzerine etkileri.

Ikinci (2014) also reported that in parallel with the increase in GA_3 dose, germination rate of argan seeds increased and 2000 ppm GA_3 was the best treatment. Also, positive effect of gibberellic acid

on germination of guava seeds has been reported by Kumar *et al.* (1991). The germination of GA_3 applied seeds in the other two media did not followed a regular course. When compared to the control, the germination rate in the mixture of peat and perlite decreased at lower doses of GA_3, and then increased at 2000 mg/l GA_3. However, there was a very irregular germination in the perlite. The control and GA_3 applied seeds of *D. orientalis* had the same average germination percentage in three media (17% for peat and peat+perlite, 18% for perlite).

The effects on germination of hot water treatments and the interactions between media and hot water treatments weren't found to have significant (Table 3). However, hot water treatment at 50°C for 2 minutes slightly increased germination rate of the seeds compared to the control, higher temperatures decreased the germination rate (Figure 3). In this study, many seeds treated with hot water rotted and died, indicating that they were very sensitive to high temperature.

Table 3. Effects of different hot water pre-sowing treatments and growing medium on germination of *D. orientalis* seeds in the greenhouse conditions.
Çizelge 3. Sera koşullarında *D. orientalis* tohumlarının çimlenmesi üzerine sıcak su ön uygulamaları ve yetiştirme ortamlarının etkileri.

Hot Water (HW) (°C)	Germination / Çimlenme (%)			Mean
	Growing medium			
	Peat	P+P	Perlite	
Control	23 a[1]	37 a	20 b	27 A[x]
50	17 a	33 a	43 a	31 A
60	13 a	30 a	0 b	14 A
70	27 a	33 a	6 b	22 A
Mean	20 AB	33 A	17 B	

F values: Media **, HW: NS, Media x HW: NS.
Means in the same columns and lines followed by the different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).
**, NS: Significant at 0.05 and non-significant, respectively. CV (%): 73.36
F değerleri: Ortam: **, Sıcak su: ÖD, Ortam x Sıcak su: ÖD
Aynı satır veya sütunda farklı harfle gösterilen ortalamalar Duncan Testine gore istatistiksel olarak ($p \leq 0,05$) farklıdır.
**, ÖD: sırasıyla istatistiksel olarak 0,05 düzeyinde önemli ve önemli değil.
VK (%): 73,36.

Sujatha and Manjappa (2015) reported that similarly hot water treatment (80°C for 10 minutes) was reduced the germination percentage of *Melia azedarach* significantly when compared to all other treatments. This might be due to lethal effect of higher temperature of water on embryo. Similar results reporting lethal effect of hot water was reported by Khantwal *et al.* (2008) who reported as low as 3% germination of *Bauhinia variegate* L. seeds in hot water treatment. Also, *Vitex doniana* seeds have a lower germination rate at high temperatures in hot water (Salisu and Jiya, 2016). Media were significantly ($P<0.05$) effective on seed germination rates. The tested seeds had the highest germination rates with 33% in the mixture of peat and perlite.

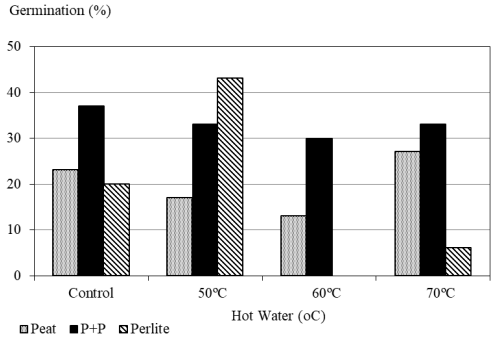

Figure 3. Effects on germination of *D. orientalis* seeds of the interactions between hot water and medium.
Şekil 3. Sıcak su ve ortamlar arasındaki interaksiyonların *D. orientalis* tohumlarının çimlenmesi üzerine etkileri.

Low temperature treatments ($P<0.01$) and its periods ($P<0.05$) had significant effect on germination. In this study, the control seeds (50%) had the highest germination rate (Table 4).

Low temperature treatments reduced germination rate of the seeds compared to the control seeds (Figure 4). This may be related to the growth of the species in coastal line and 20 m in height. Leo (2013) reported that similarly the cold stratification reduced the rate of seed germination in only one out of 28 species examined when they compared the control seeds.

Germination rate of the seeds were higher in the mixture of peat and perlite than that in the others. But there was no significant difference between the media. There was no significant difference in germination rates of the interactions between media, low temperatures and durations. The best

result of the experiment was obtained with 67% germination for the control seeds sown in the mixture of peat and perlite.

Table 4. Effects of different low temperature pre-sowing treatments and growing medium on germination of *D. orientalis* seeds in the greenhouse conditions.
Çizelge 4. Sera koşullarında *D. orientalis* tohumlarının çimlenmesi üzerine düşük sıcaklık ön uygulamaları ve yetiştirme ortamlarının etkileri.

Low Temp (LT) (°C)	Period (Days)	Germination/ Çimlenme (%) Growing medium Peat	P+P	Perlite	Mean
C	0	0.47 a[1]	0.67 a	0.37 a	0.50 A[x]
5	20	0.23 ab	0.23 b	0.30 a	0.23 B
	40	0.20 b	0.27 b	0.17 a	
10	20	0.36 ab	0.40 b	0.20 a	0.25 B
	40	0.23 b	0.17 b	0.13 a	
Mean		0.30 A	0.35 A	0.23 A	

F values: Period: **, LT: ***, Media: NS, Media x LT x Period: NS.
Means in the same collums and lines followed by the different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).
, *, NS; Significant at 0.05, 0.01 and non-significant, respectively. CV (%): 56.82.
F değerleri: Süre: **, Düşük sıcaklık: ***, Ortam x Düşük sıcaklık x Süre: ÖD.
Aynı satır veya sütunda farklı harfle gösterilen ortalamalar Duncan Testine gore istatistiksel olarak ($p \leq 0.05$) farklıdır.
, *, ÖD; sırasıyla istatistiksel olarak 0.05, 0.01 düzeyinde önemli ve ÖD
VK (%): 56,82.

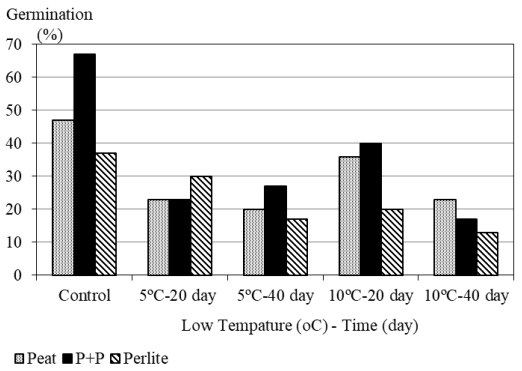

Figure 4. Effects on germination of *D. orientalis* seeds of the interactions between low temperature and medium.
Şekil 4. Düşük sıcaklık ve ortamlar arasındaki interaksiyonların *D. orientalis* tohumlarının çimlenmesi üzerine etkileri.

In almost all greenhouse seed germination studies, the lowest germination rate was obtained from the perlite medium. Seedling development was also poor in perlite. Peat and the mixture of perlite and peat were better than perlite in experiments.

In vitro Seed Germination

In vitro germination of seeds subjected to GA_3, hot water and low temperature pre-treatments were not statistically significant.

In vitro gibberellic acid pre-treatments gave a similar result to the nursery GA_3 treatments. When 10, 50 and 100 mg/l GA_3 concentrations were compared to the control, GA_3 prevented seed germination and caused a lower germination rate (Figure 5).

However the germination rate at 250 mg/l (50%), which was the highest dose of *in vitro* GA_3 treatments, was better than control and other GA_3 treatments. Srivastava *et al.* (2011) reported that GA_3 applications completely prevented seed germination in *Aconitum heterophyllum* and could not improve the seed germination even at low temperature. It is known that treatment of *Prunus mahaleb* seeds with gibberellic acid has been reported to overcome dormancy and ensure uniform germination (Al-Absi, 2010).

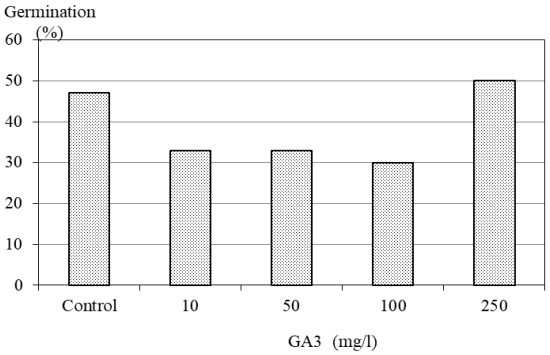

Figure 5. Effects of different GA_3 pre-sowing treatments on germination of *D. orientalis* seeds under *in vitro* conditions.
Şekil 5. *In vitro* koşullar altında *D. orientalis* tohumlarının çimlenmesi üzerine farklı GA_3 ön uygulamalarının etkileri.

After hot water pre-sowing treatment, *in vitro* germination graph of the seeds was similar to in vivo culture. Germination rate of seeds soaked in 50°C hot water was 40% and found to be higher than control (Figure 6). Increasing the application temperature caused reduction in the rate of germination. Hot water treatment at 70°C completely prevented seed germination. Sakhanokho (2009) reported that germination rates of 54% (*ex vitro*) and 95% (*in vitro*) were achieved when *Hibiscus dasycalyx* seeds were treated with hot water for 5 min, but exposing the seeds for 10, 15, or 20 min produced poor results in *H. acetosella* and *H. dasycalyx* as hot water

scarification appeared to result in severe injury or death of the embryos.

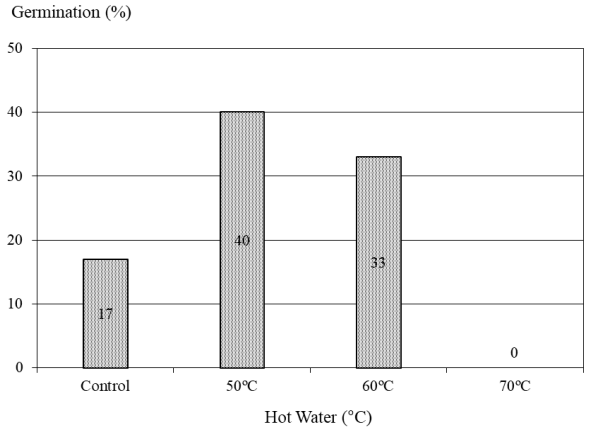

Figure 6. Effects of different hot water pre-sowing treatments on germination of *D. orientalis* seeds under *in vitro* conditions.
Şekil 6. *In vitro* koşullar altında *D. orientalis* tohumlarının çimlenmesi üzerine farklı sıcak su ön uygulamalarının etkileri.

In vitro low temperature pre-sowing treatments gave a different result from the nursery low temperature treatments. The highest germination rate with 47% was determined from seeds stored at 5°C for 30 days (Figure 7).

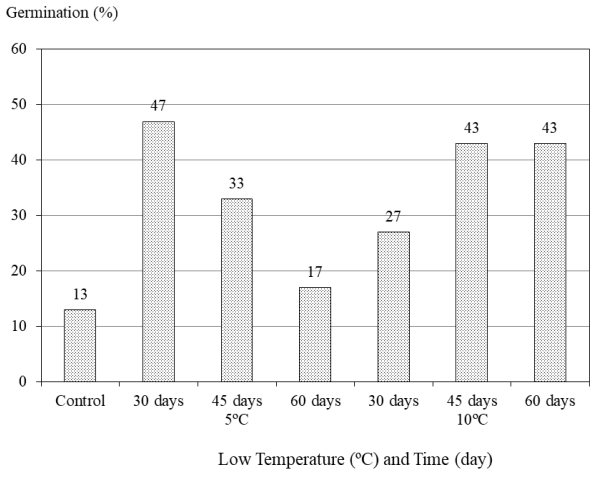

Figure 7. Effects of different low temperature pre-sowing treatments on germination of *D. orientalis* seeds under *in vitro* conditions.
Şekil 7. *In vitro* koşullar altında *D. orientalis* tohumlarının çimlenmesi üzerine farklı düşük sıcaklık ön uygulamalarının etkileri.

It was more successful than that seeds stored at 10°C and the control. Similarly, Srivastava *et al.* (2011) reported that different low temperature applications of *A. heterophyllum* seeds had a higher germination rate than the control and that the highest germination rates were taken from the seeds that were kept at 15 ° C for 8 weeks.

In the study, it was determined that the germination rate increased at 5°C as the storage period decreased, while at 10°C, the germination rate of the seeds increased as the storage period increased.

CONCLUSION

This study was carried out in order to determine the propagation method of *D. orientalis* which is not studied much yet. For this purpose, the possibility of propagation by seed and cutting was investigated. Propagation by cutting was more unsuccessful than by seed. However, 28% rooting success could be achieved by cutting. This may be due to the thinness of cutting structure. The propagation by seed was carried out both in the nursery and *in vitro* conditions. Before sowing the seeds of *D. orientalis*, some pre-sowing treatments (GA_3, hot water and low temperature) were treated in both greenhouse and *in vitro*. Seeds showed mostly a maximum germination rate of 40% to 50% in all experiments in both of them.

In this study, perlite medium was unsuccessful in terms of both rooting rate and germination rate. Similarly, seedling development was also poor in perlite medium. This is due to the fact that perlite does not retain water as much as peat. For this reason, perlite must be watered frequently. Peat is known to work well with other components to provide better physical properties which are necessary for optimum plant growth. Therefore, peat or perlite and peat mixture as medium in the experiments was better than perlite.

As a result, *D. orientalis* can be propagated both by seed and by cutting. But it seems more appropriate to propagate it with seed. Along with planning new studies to increase the germination rate of seeds of this species, alternative production methods should be also tried.

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