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Effects of Different Treatments on Germination Capacity of Hawthorn (Crataegus spp.) Seeds

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Hawthorn (Crataegus spp.) species, Seed priming, Germination rate

Abstract

Hawthorn (*Crataegus* spp.), one of the wild fruit species that stands out with its various uses, is used as a medicinal plant and is important in the pharmaceutical field because its flowers and fruits have blood pressure reducing, heart regulating and strengthening effects. In addition, in recent years, it has become one of the most desired fruits for fresh consumption and orchards have started to be established in Türkiye. The material of the study consisted of the genotypes of three different hawthorn species (*Crataegus pseudoheterophylla, Crataegus × sinaica and Crataegus rhipidophylla var. rhipidophylla*), which are naturally found in Sivas, Bolu, Kütahya and Karabük provinces and are known to be very difficult to propagate by vegetative methods. The aim of the study is to investigate the effects of different treatments on seed germination in the seeds of these species. Crataegus monogyna species was used as a control in the study. In terms of thousand seed weight, *Crataegus monogyna* with 160.25 g, *Crataegus pseudoheterophylla* with 120.30 g and *Crataegus rhipidophylla var. rhipidophylla* with 85.89 g showed the highest values. In terms of seed occupancy rate, *Crataegus rhipidophylla var. rhipidophylla* (93.33%) and *Crataegus monogyna* (63.33%) genotypes stood out. The highest germination rates were obtained from *Crataegus monogyna* (51.66%) and *Crataegus rhipidophylla var. rhipidophyla* (43.33%) with lye + seaweed soaking treatment. In Crataegus × sinaica, 31.66% germination rate was obtained with gibberellic acid treatment. It is predicted that the results obtained will contribute to the selection of appropriate methods according to the species to overcome germination difficulties in hawthorn, to expand the commercial cultivation and utilization areas of hawthorn and to the conservation of these important genetic resources.

Alıç (Crataegus spp.) Tohumlarının Çimlenme Kapasitesi Üzerine Farklı Uygulamaların Etkisi

Anahtar Kelimeler

Alıç (Crataegus spp.) türü, Tohum uygulamaları, Çimlenme oranı

Özet

Farklı kullanım alanlarıyla öne çıkan yabani meyve türlerinden birisi olan alıç (*Crataegus* spp.), çiçek ve meyveleri, tansiyon düşürücü, kalp düzenleyici ve kuvvetlendirici etkiye sahip olması nedeniyle tıbbi bitki olarak kullanılmakta ve farmasotik alanda önem taşımaktadır. Ayrıca son yıllarda taze tüketim için en çok aranan meyvelerden biri haline gelmiş ve Türkiye'de meyve bahçeleri kurulmaya başlamıştır. Araştırmanın materyalini, Sivas, Bolu, Kütahya ve Karabük illerinde doğal olarak bulunan ve vejetatif yöntemler ile çoğaltılmasının oldukça zor olduğu bilinen üç farklı alıç türünün (*Crataegus pseudoheterophylla, Crataegus × sinaica ve Crataegus rhipidophylla var. rhipidophylla*) genotipleri oluşturmuştur. Araştırmanın amacı bu türlere ait tohumlarda farklı uygulamaların tohum çimlenmesi üzerine etkilerini araştırmaktır. Araştırmada kontrol olarak *Crataegus monogyna* türü kullanılmıştır. Bin tane ağırlığı yönünden 160.25 g ile *C. monogyna*, 120.30 g ile *C. pseudoheterophylla* ve 85.89 g ile *C. rhipidophylla var. rhipidophylla* en yüksek değerleri vermişlerdir. Çekirdek doluluk oranı yönünden ise *C. rhipidophylla var. rhipidophylla* (%93.33) ve *C. monogyna* (%63.33) genotipleri ön plana çıkmıştır. En yüksek çimlenme oranları küllü su + deniz yosununda bekletme uygulaması ile *C. monogyna* (%51.66) ve *C. rhipidophylla var. rhipidophylla* (%43.33) türlerinden elde edilmiştir. *Crataegus × sinaica* türünde gibberellik asit uygulaması ile %31.66 çimlenme oranı elde edilmiştir. Elde edilen sonuçlar, alıçta çimlenme engellerinin aşılması için türe göre uygun yöntem seçilmesine, alıcın ticari yetiştiriciliği ve kullanım alanlarının genişletilmesine ve bu önemli genetik kaynakların korunmasına katkıda bulunacağı öngörülmektedir.

1. INTRODUCTION

Hawthorn (Crataegus spp.) is widely distributed in the northern hemisphere and is represented by more than 150 species in the New World and 60 species in the Old World (Dönmez, 2014). Hawthorn is found in both shrub and single tree forms and is widespread in Western Asia, North America and Europe. It is one of the oldest plants used for medicinal purposes as well as fruit and decorative purposes (Ercisli, 2004, Yılmaz et al., 2010; Çalışkan et al., 2016). Türkiye is an important gene center for hawthorn species. Including the recently described hawthorn species, there are 25 hawthorn species in Turkey (Dönmez, 2014). Hawthorn grows naturally in mountain regions, bushland and rocky areas in Türkiye and no cultural practices is applied to this native plant. The most widespread hawthorn species in Turkey is Crataegus monogyna, while Crataegus orientalis, Crataegus oxyacantha, and Crataegus aronia are also common species. In different regions of Turkey, hawthorn is known as alıç, aluç, halıç, yemişen, wild rose, June or sour medlar (Balta et al., 2015; Çalışkan et al., 2016).

Hawthorn is a type of fruit that is naturally distributed in almost every region of Turkey. Hawthorn, whose modern hawthorn orchards is carried out in the province of Hatay, is consumed as collected from nature in provinces such as Gümüşhane, Tokat, Bolu, Aksaray, Eskişehir, Kütahya, Ankara, Yozgat, Antalya, Çorum, Malatya, Nevşehir, Niğde, Sivas, Muş, Siirt. In addition, there is a potential to expand hawthorn cultivation in Mersin, Aksaray, Osmaniye, Adıyaman and Malatya provinces (Çalışkan et al., 2018)

A most important limiting factor for the hawthorn growing is propagation. Vegetative propagation of hawthorn species is not a usefully method because of their rooting percentage is very lower. Moreover, even if hawthorn is successfully propagated under in vitro conditions, root shoot production is insufficient under in vitro conditions (Güney et al., 2020). At the present time, rootstock production is curried out with seeds, and then grafting and budding methods are used for new plants (Çalışkan and Karaman, 2018; Kacal et al., 2022).

One of the natural processes for propagating hawthorn plants is seed propagation. A new plant is formed from the seeds of the plants that fall to the ground. Seed propagation is a simple method, but it is a technical process with many problems (Kaşka and Yılmaz, 1987). There are many internal and external factors affecting seed germination. These factors include the inhibition of water uptake by the seed coat (mechanically affecting the embryonic development), dormancy of the embryo and the presence of inhibitors that prevent seed germination (Diaz and Martin, 1972).

In general, mature seeds of many tree and shrub species do not germinate despite suitable environmental conditions such as temperature, humidity, oxygen and light. This phenomenon is called dormancy. Dormant seeds do not germinate right after harvesting unless they are applied some special treatments. Although dormancy in seeds is a very important feature for the survival of species, it is not desirable for growers and breeders. Because the germination of dormant seeds sown without any treatment takes a long time and occurs irregularly. This causes long duration of land utilization in nurseries, loss of time, inhomogeneous seedlings of different ages and sizes, and increase in cost (Tanrıverdi, 1975).

The treatments required to break dormancy vary from species to species and even between seeds of different origin of the same species. In order to break dormancy, stratification, removing or breaking the seed coat, soaking in water, using growth regulators, washing, drying, heat and light treatments, mechanical stratification and acid treatment or a combination of one or more of these treatments have been used (Tanrıverdi, 1975; Hartman et al., 2002). It has been reported that cold folding after acid treatment or hot water soaking is effective for both seeds with hard coats and embryo dormancy (Hartman et al., 2002).

Although it has been reported that hawthorn plants are very difficult to propagate by tissue culture (Gökbunar, 2007) and cuttings (Ünsal, 2012), it has been possible to partially produce them by tissue culture with the recent advances. As a matter of fact, seed propagation (Çalışkan et al., 2020) or tissue culture (Nas et al., 2012) may be preferred in rootstock production for hawthorn cultivation. However, in practical terms, it is observed that plants propagated by tissue culture cannot survive in difficult field conditions and serious losses are observed. On the other hand, hawthorns grafted on seed propagated rootstocks are better adapted to extreme field conditions.

In parallel with global warming and drought, the importance of drought-resistant rootstocks and ornamental plants that require less irrigation is increasing. In this case, it is important to conserve drought-resistant species such as hawthorn and it is thought that the demand in this respect will increase in the near future (Bektaş et al., 2017). However, it is difficult to meet the demand in a short period of time, as insufficient research has been carried out on hawthorn cultivation, selection of suitable types and especially propagation.

In recent years, the increasing demand for functional products and the interest in alternative medicine has led to the demand for many products such as fruits, flowers and roots in hawthorns as in many wild fruit species (Özçelik, 2016). In line with this request, the producers have focused on producing hawthorn genotypes selected for their superior fruit characteristics by grafting them onto the rootstock.

However, in hawthorns, which have problems in seed germination in terms of obtaining rootstocks, the primary objective of this study is to obtain vigorous, homogeneous and compatible rootstocks in a short time. For these reasons, this study was aimed to determine the effects of different treatments on the germination rate of hawthorn seeds of different hawthorn species (*Crataegus* spp.) in order to facilitate the production of hawthorn seedlings until the problems in tissue culture plants are solved. In this regard, the effects of different treatments on the germination performance of hawthorn seeds of different hawthorn species, which have problems in seed propagation, taken from Sivas, Bolu, Kütahya and Karabük provinces, which are known to have high hawthorn populations, were determined and the germinated seeds were taken to field conditions and preserved as genetic resources.

2. MATERIAL AND METHODS

2.1. Material

The study was conducted between September/2021 and August/2023 in hawthorn plants growing naturally in Sivas, Bolu, Kütahya and Karabük provinces and districts (Figure 1). In this study, which was carried out to identify different hawthorn genotypes, each hawthorn plant selected was considered as a genotype. These provinces were selected because hawthorn grows naturally in mountainous areas and shows a diverse population. In addition, the species used in the study were determined by species identification in previous studies.

Figure 1. Provinces where hawthorn genotypes used in the study were collected.

2.2. Methods

The germination tests were established by collecting seeds of 3 different hawthorn species (*Crataegus pseudoheterophylla*, *Crataegus* × *sinaica and Crataegus rhipidophylla var rhipidophylla*) from Sivas, Bolu, Kütahya and Karabük provinces (minimum 1 genotype for each species). Hawthorn fruits were collected from the areas where the species are naturally distributed and the fruits were crushed in a container to separate the fruit flesh and seeds. Then, this container was filled with water and the fruit flesh floating in the water was removed, and the seeds remaining at the bottom were washed with plenty of water and completely cleaned from the fruit flesh. The cleaned and separated seeds were left to dry in a shady place with good air flow. After drying for about three days, the seeds were stored at 5±1°C in zipper bags until pre-treatment and sowing time. In the seed germination test, the seeds of each species were subjected to the following treatments. The preservation of the propagated material was carried out in the greenhouse and garden of the Erciyes University Faculty of Agriculture.

Thousand seed weight: The 1000 grain weight of the seeds was obtained by measuring the weights of 10 samples of 100 seeds taken randomly, averaging them and multiplying by 10 (ISTA, 1993).

Seed occupancy rate: The occupancy rates were determined by breaking 3x100 seeds randomly selected from the seeds.

Dormancy breaking treatments (Bujarska-Borkowska, 2006):

- *Warm stratification:* Keeping the seeds at 35 °C for 12 weeks followed by cold stratification (keeping them at 4-5 °C for 12 weeks).
- *Scarification I:* After soaking in 96% sulfuric acid (H_2SO_4) for 2 hours, warm treatment (4 weeks at 35 °C) was applied.
- *Scarification II:* After soaking in 96% sulfuric acid (H_2SO_4) for 2 hours, cold treatment (16 weeks at 3°C) was applied.
- After sulfuric acid applications, the seeds were washed with plenty of water.
- *Mechanical abrasion I:* After sandpaper application, warm stratification (16 weeks at 35 °C) followed by cold stratification (16 weeks at 5 °C) was performed.

- *Mechanical abrasion II:* After application with sandpaper, they were kept in warm water for 24 hours.
- *Gibberellic acid treatment:* The seeds were treated with a dose of 1000 ppm of GA3.
- *Soaking in water:* The seeds were kept in water at room temperature for 24 hours.
- *Soaking in lye + seaweed:* Oak ash was used for soaking in lye and the seeds were treated in a solution containing 10% oak ash (100 g/l). In these seeds, 6 days soaking in lye, + 1 day soaking in water containing 1/5000 seaweed for 1 day were applied.
- *Soaking in seaweed:* In the commercial product Maxicrop, which contains seaweed, soaking in water containing 1/500 of seaweed (3 days) were applied.
- *Control:* Seeds were used without any treatments.

The stratification was carried out in wooden boxes by placing one layer of moist sand and one layer of seeds in moistened cloth bags on top of each other. In the treatment, the sand was moistened at 5±1°C (humidity was checked weekly). After the stratification treatments, the seeds were germinated in petri dishes at 30°C. The germination of the seeds was monitored twice a week for 75 days.

After germination was completed, germination percentages and germination rates of the seeds sown for each pretreatment applied to the seeds were determined. The experiment was established according to the randomized block design with 3 repeats and 15 seeds in each block. The germination of the seeds was checked twice a week in all treatments. The data of the study were evaluated using the JMP Pro 14 (SAS Institute Inc., Cary, NC, USA) statistical package program. The difference between the means was determined at the 5% significance level according to the Tukey multiple comparison method.

3. RESULTS AND DISCUSSION

Hawthorn seeds are often considered as waste material like many other fruit pits, especially in the food industry (Can et al., 2010). However, recent research has revealed that hawthorn seeds possess a variety of pharmacological properties, including pain killer, blood pressure reducing, anti-inflammatory and antioxidant effects with rich flavonoids (Peng et al., 2016). The determination of seed traits, which usually depends on genotype and environmental conditions, has become increasingly important, especially in breeding programs (Muradoğlu et al., 2021). Significant differences were found in the investigated hawthorn genotypes in terms of seed characteristics. Thousand seed weight (g) and seed occupancy rate (%) of hawthorn species are given in Table 1. The highest value in terms of thousand seed weight (g) was obtained from *Crataegus monogyna* species with 160.25 g. It was followed *by C. pseudoheterophylla* with 120.30 g, *Crataegus rhipidophylla* var. *rhipidophyll* with 85.89 g and *Crataegus × sinaica* with 82.05 g. In the literature, the thousand seed weight of *Crataegus monogyna* species was reported as 98 g (Gardens, 2014), 133 g (Khadivi et al., 2019) and 280 g (Kheloufi et al., 2019). The highest value in terms of seed occupancy rate was obtained from *Crataegus rhipidophylla* var. *rhipidophyll* with 93.33%. This was followed by *Crataegus monogyna* with 63.33%, *Crataegus pseudoheterophylla* with 53.33% and *Crataegus × sinaica* with 40% occupancy rate. Yahyaoğlu et al. (2006) reported occupancy rates as 89% in *Crataegus monogyna* subsp. *azarella*, 88% in *Crataegus microphylla* and *Crataegus pontica*, 87% in *Crataegus monogyna* and 86% in *Crataegus pseudoheterophylla*. Yıldırım (2018) reported seed occupancy rates as 45% in *Crataegus pontica* and 44% in *Crataegus monogyna*.

Table 1. Thousand seed weight (g) and seed occupancy rate (%) of hawthorn species used in the study

Species	Thousand seed weight (g)	Seed occupancy rate (%)
Crataegus pseudoheterophylla	120.30	53.33
Crataegus × sinaica	82.05	40
Crataegus rhipidophylla var. *rhipidophyll*	85.89	93.33
Crataegus monogyna	160.25	63.33

Hawthorn seeds have serious physiological germination inhibitions due to hard seed coat and embryo dormancy (Mohammed, 2023). In order to overcome these problems, certain treatments should be applied to hawthorn seeds before sowing. In general, warm and cold stratification treatments are recommended by researchers to eliminate germination barriers caused by immature hawthorn seed embryos (Göktürk and Yıldırım, 2020). The effect of different seed treatments on germination in hawthorn species is given in Table 2. There was no significant effect of warm stratification on germination in hawthorn species. Other seed treatments had significant effects on germination rates. The highest germination rate of *Crataegus monogyna* species with 51.66% was obtained from the treatment of soaking in lye + seaweed. *Crataegus rhipidophylla* var. *rhipidophyll* species had the second highest germination rate with 43.33%. In addition, the highest germination rate of

Crataegus pseudoheterophylla species was obtained from the treatment of soaking in lye + seaweed with 28.33%. Göktürk and Yılmaz (2015) obtained 74.44% germination rate in *Crataegus orientalis* species by soaking in lye. Subaşı (2018) reported that lye application promoted germination by 2.67% in *Crataegus orientalis* species. Kızılaslan (2019) reported that the pretreatment of soaking in lye increased germination by 23.34% in *Crataegus pontica* species and that the germination rates increased with the longer soaking time. In *C. pseudoheterophylla* species, the highest values were obtained from mechanical scarification II (16.66%), mechanical scarification I (10%) and gibberellic acid treatments (8.33%) after the application of lye + seaweed soaking. The treatments used to increase the germination rate and speed can be applied singly or in various combinations with some other agents (Karakurt et al., 2010). Tabari Kochaksaraei (2015) obtained 19.7% germination rate with mechanical scarification application in *Crataegus pseudoheterophylla* species. Göktürk and Yıldırım (2020) obtained 35.29% germination rate with mechanical scarification.

The highest germination rate of *Crataegus* × *sinaica* was obtained from gibberellic acid treatment with 31.66%. This was followed by mechanical abrasion I with 23.33%, lye + seaweed soaking with 21.66% and mechanical abrasion II with 13.33%. In *Crataegus rhipidophylla* var. *rhipidophyll* species, the highest germination rate was obtained from water soaking with 15% after soaking in lye + seaweed and mechanical abrasion II treatments. This was followed by seaweed soaking treatment with 11.66% and gibberellic acid treatment with 8.33%. In *Crataegus monogyna* species, the highest germination rate was obtained from the mechanical abrasion II treatment with 25% after the ashy water + seaweed soaking treatment. This was followed by gibberellic acid treatment and seaweed soaking treatments with 18.33%. Seeds, which are usually hard-shelled, are usually covered with a thick and impermeable layer. In order to increase germination in such seeds, they are treated with acids to erode the hard coat (Karakurt et al., 2010). Göktürk and Yıldırım (2020) obtained the highest germination rate in *Crataegus monogyna* species with 64.98% from sulfuric acid treatment. In many studies, it has been reported that gibberellic acid application increases germination in many species of the Rosaceae family (Ghayyad et al. 2010) and contributes to overcome physiological dormancy in seeds with dormant embryos (Hartmann et al. 2010). Ahmadloo et al. (2017) reported that gibberellic acid application in *Crataegus pseudoheterophylla* species increased germination by 9% in shelled seeds and 59.7% in unshelled seeds. Following the fruit harvest, the hard-impermeable endocarp surrounding the hawthorn seeds was broken. Subsequently, 3000 ppm GA3 was applied to the seeds, as a result of this application up to 60% germination within approximately one month (Çalışkan et al., 2020).

Table 2. Effect of different seed treatments on germination in hawthorn species (%)

	Treatments	*Crataegus pseudoheterophylla*	*Crataegus* × *sinaica*	*Crataegus rhipidophylla var. rhipidophyll*	*Crataegus monogyna*
1	Warm stratification	0.00d	0.00d	0.00d	0.00c
2	Scarification I	1.66d	0.00d	3.33cd	5.00c
3	Scarification II	1.66d	3.33cd	6.67cd	3.33c
4	Mechanical abrasion I	10.00c	23.33ab	5.00cd	0.00c
5	Mechanical abrasion II	16.66b	13.33bc	23.33b	25.00b
6	Gibberellic acid treatment	8.33c	31.66a	8.33cd	18.33b
7	Soaking in water	0.00d	3.33cd	15.00bc	0.00c
8	Soaking in lye + seaweed	28.33a	21.66ab	43.33a	51.66a
9	Soaking in seaweed	5.00cd	8.33cd	11.66cd	18.33b
10	Control	0.00d	0.00d	0.00d	0.00c

* The difference between the averages indicated by different letters letter in the same column is significant ($p < 0.05$)

CONCLUSION

This study was carried out on hawthorn species collected from Sivas, Bolu, Kütahya and Karabük provinces where hawthorn populations are known to be dense, and the effects of different treatments on the germination performance of hawthorn seeds were determined. The outstanding genotypes in seed traits were determined. In terms of thousand seed weight, *Crataegus monogyna*, *Crataegus pseudoheterophylla* and *Crataegus rhipidophylla* var. *rhipidophylla* were the prominent species, respectively. In addition, the highest seed occupancy rates were obtained from genotypes belonging to *Crataegus rhipidophylla* var. *rhipidophylla* and *Crataegus monogyna* species. The highest seed germination rates were obtained from *Crataegus monogyna* and *Crataegus rhipidophylla* var. *rhipidophyll* with lye + seaweed soaking treatment. High germination rate was obtained with gibberellic acid treatment in *Crataegus* × *sinaica* species. The results obtained will contribute to the selection of appropriate methods to solve germination problems in hawthorn according to species. In addition, it is predicted that the genotypes obtained can be used as breeding material in further studies, increasing the commercial cultivation and usage areas of hawthorn, protecting these important genetic resources and making important contributions to the literature.

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Conflicts of Interest

The authors declare no conflict of interest.

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