

Determination of Seed Morphologies and Effect of Pretreatments on Germination of *Crataegus monogyna* (Jac.) and *Crataegus azarolus* var. *pontica* (K. Koch) K. I. Chr Seeds

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Abstract

Aim of the study: This study was conducted to determine seed morphologies and the effects of pretreatments on germination of common hawthorn (*Crataegus monogyna* Jac.) and azarole (*Crataegus azarolus* var. *pontica* (K. Koch.) K. I. Chr) seeds.

Material and methods: Ripe fruits of the common hawthorn and azarole seeds were collected from trees growing in Hatila Valley and Pamukcular Village in September 2016 in Artvin, Turkey. The seeds were subjected to varying durations of ash solution, sulfuric acid, hydrogen peroxide, ethanol and combinations of ash solution and sulfuric acid pretreatments. The seeds were sown according to a randomized complete block design with four replications.

Main results: Germination of azarole seeds (37.31%) was higher than that of common hawthorn seeds (16.53%). The ash solution pretreatments did not affect the germination of the common hawthorn seeds. However, it was found to be more effective ($p < 0.05$) on germination of azarole seeds. The highest germination percentage (64.98%) of azarole seeds were found in seeds that had been treated in sulfuric acid for 6 hours with ash solution for 144 hours.

Highlights: In order to remove the seed dormancy of azarole seeds, an ash solution treatment can be applied together or separately with sulfuric acid.

Keywords: Acidic Chemicals, Alkaline Ash Solution, Azarole, Common Hawthorn, Seed Dormancy.

Crataegus monogyna (Jac.) ve *Crataegus azarolus* var. *pontica* ((K. Koch) K. I. Chr) Tohumlarının Morfolojilerinin ve Ön İşlemlerin Tohumlarının Çimlenmesi Üzerine Etkisinin Belirlenmesi

Öz

Çalışmanın amacı: Bu çalışma yemişen (*Crataegus monogyna* Jac.) ve müzmüldek (*Crataegus azarolus* var. *pontica* (K. Koch.) K.I. Chr) tohumlarının morfolojilerini ve ön işlemlerin tohumların çimlenmesi üzerindeki etkilerini belirlemek amacıyla yapılmıştır.

Materyal ve yöntem: Yemişen ve müzmüldek olgun meyveleri, Artvin ilinde (Türkiye) Hatila Vadisi ve Yusufeli Pamukçular köyünde yetişen ağaçlardan Eylül 2016'da toplanmıştır. Tohumlar, farklı sürelerde kül çözeltilisinde bekletme, sülfürik asit hidrojen peroksit ve etanol ile kimyasal aşındırma ve kül çözeltilisinde bekletme ile sülfürik asitte kimyasal aşındırma ön işlemlerinin kombinasyonlarına tabi tutulmuştur. Tohumlar, dört tekrarlı tesadüfi tam blok deneme desenine göre ekilmiştir.

Temel sonuçlar: Müzmüldek tohumlarının çimlenme yüzdesi (% 37.31) yemişen tohumlarının çimlenme yüzdesinden (% 16.53) daha yüksektir. Kül çözeltilisinde bekletme, yemişen tohumlarının çimlenmesini etkilememiş, ancak müzmüldek tohumlarının çimlenmesinde önemli ($p < 0.05$) etkisi olmuştur. Müzmüldek tohumlarında en yüksek çimlenme yüzdesi (% 64.98) 6 saat sülfürik asitte zedeleme ve 144 saat kül çözeltilisinde bekletme işlemlerinin birlikte uygulanmasından elde edilmiştir.

Araştırma vurguları: Alkali kül çözeltisi müzmüldek tohumlarının çimlenme engelini gidermek amacıyla sülfürik asitle zedeleme işlemiyle birlikte veya ayrı olarak uygulanabilir.

Anahtar Kelimeler: Asidik Kimyasallar, Alkali Kül Çözeltisi, Müzmüldek, Yemişen, Çimlenme Engeli



Introduction

Seeds of the *Crataegus* (L) species exhibit both endogenous (related to immature embryos) and exogenous (related to seed coat properties) dormancy. In general, cold and warm stratification in controlled conditions is suggested to break endogenous dormancy (Bujarska-Borkowska, 2007), while mechanical and chemical scarifications are recommended for enabling exogenous dormancy (Morgenson, 2000; Yahyaoglu et al., 2006). However, for some *Crataegus* species, chemical scarification was not found to be beneficial for germination (Morgenson, 2000).

Exogenous dormancy of *Crataegus* seeds is caused by the presence of hard and thick seed coats, which affects permeability. The thickness of seed coats varies among *Crataegus* species and even within the same species (Gokturk et al., 2017). Owing to this variability in seed coat thickness, scarification in sulfuric acid pretreatments are required at different durations. Sulfuric acid is a very strong acid, thus, scarification in sulfuric acid treatments causes seed coats to become thinner. This degeneration of seed coats can be identified with scarification rates which increase with the duration of scarification in sulfuric acid (Gokturk et al., 2017). Immersion time of seeds for scarification in sulfuric acid of *Crataegus* species varies between one to three hours (Brinkman, 1974; St. Jhon, 1982; Young & Young, 1992; Bujarska-Borkowska 2002-2006; Yahyaoglu et al., 2006). It increases up to six hours for some *Crataegus* species with thicker seed coats (Dirr and Heuser, 1987).

Crataegus genus is represented with a total of 27 taxa, including 16 species, three subspecies, six hybrid and six varieties in Turkey (Özkan et al., 2014). Two of these species are azarolus (*Crataegus azarolus* L. var. *pontica* (K. Koch) K.I.Chr.) and common hawthorn (*Crataegus monogyna* Jacq.). The general distribution areas of azarolus are in Georgia, Northern Iran and east of the Caspian Sea (Transcaspiya). In Turkey, there are rare species that are spread throughout Coruh, Erzurum and Nevşehir (Browicz, 1972; Christensen & Zielinski 2008; Dönmez, 2004). Common hawthorn spreads generally in Europe, Cyprus, Syria

and Northern Iraq, and it is also common in Turkey (Özkan et al., 2014).

Common hawthorn and azarolus are both in the form of thorny bushes and trees that grow up to 10 m. Common hawthorn fruits are almost spherical or in an egg-like formation, 6-10 mm in diameter, red or orangish red colored, with 1-2 nutlets. Azarole fruits are yellowish or orange colored, spherical, 12-25 mm in diameter, with 2-3 nutlets (Özkan et al., 2014). The fruits of *Crataegus* are edible with a high nutritional value including large amounts of Ca, P, K, Mg and Fe mineral substances (Ozcan et al., 2005), and a small quantity of them have more vitamin C than in one orange (Morton, 1981). They are important food sources for wildlife because they are commonly consumed by birds, small mammals and some ungulate animals (Shrauder, 1977). The fruit is also used in pharmacology for its medical aromatic properties. Due to these properties, the cultivation of *Crataegus* species is important. However, the desired amount of germination has not been achieved and germination has not occurred in a desired amount of time in nurseries. Dormancy of *Crataegus* seeds is causing the failure of the desired seedling production.

The aims of this study were, (1) to identify seed morphology, (2) to determine the effects of alkaline ash solution, (3) to evaluate chemical scarification (in hydrogen peroxide, ethanol, and sulfuric acid) and mechanical scarification, and (4) to determine the most effective chemical pretreatment on germination of common hawthorn and azarole seeds.

Material and Methods

Material

Common hawthorn and azarole seeds used in this study were obtained from ripe fruits (Figure 1) collected from natural distribution sites of the species during 24-26 September 2016. Common hawthorn fruits were collected from Hatila valley (aspect: SE, elevation: 1215 m, 41° 07' 00" N, 41° 37' 48" E) while azarole fruits were collected from Pamukcular village (aspect: S, elevation: 1150 m, 40° 46' 50" N, 41° 49' 40" E) in Yusufeli-Artvin, Turkey. The

pretreatments were completed in the Seed and Afforestation Laboratory of the Forestry Faculty of Artvin Coruh University. The seedling production studies were completed in the forest nursery (aspect: S, elevation: 580 m) of the Forestry Application and Research Center of Artvin Coruh University.



Figure 1. Common hawthorn (a-c) and azarole (b-d) fruits and seeds

Method

Seed Collection and Extraction of Pulp

The seeds of common hawthorn and azarole were separated from the pulp by wet maceration. The seeds were rinsed in tap water until they were completely extracted from the pulp. Empty seeds were extracted by the flotation method. Then, seeds were dried in the shade for 10 days at room temperature. Afterwards, they were stored at 0-5°C in plastic bags.

Seed Properties

Seed properties examined in this study were seed diameter, seed length, seed weight, moisture content, coat thickness, and germination percentages. A digital caliper at 0.01 mm accuracy was used to measure diameter, length and coat thickness of the seeds. Seed diameter was measured at the median region of the seed and seed length was measured as the distance between the base and the apex. Seed coat thickness was measured separately at points where the seed coat was the thickest and the thinnest (Figure 2) on seeds that were sanded to half of the seed length using a honing machine. A hundred seeds per species were used in this

process. Eight samples of 100 seeds (ISTA, 1993) were used to determine the seed weight. Ten seeds with 4-replication were used to determine the moisture content of the seeds. Seed samples were placed in a dry oven and dried at 105°C for 24 hours before they were weighed and recorded. Moisture content was calculated after dry weights of the seeds were measured by the following formula (ISTA, 1993),

$$MC = ((IW - DW) / IW) \times 100 \quad (1)$$

where MC is moisture content, IW is initial weight and DW is the dry weight.

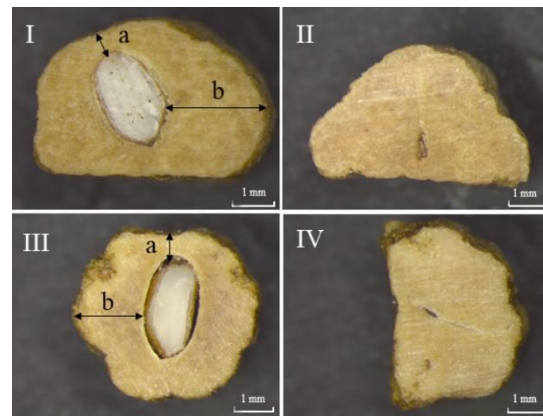


Figure 2. Thinest (a) and thickest (b) parts and full and empty seed samples of the azarole (I-II) and common hawthorn (III-IV) seeds.

Pretreatments

Seeds were subjected to oak – hornbeam mixed wood ash solution (pH: 12.5), sulfuric acid (98% H₂SO₄), hydrogen peroxide (35% H₂O₂), ethanol (99.9% C₂H₆O), mechanical scarification and a combination of sulfuric acid with ash solution treatments. Seeds were immersed in sulfuric acid in one, three and six hours according to Brinkman (1974), St. John (1982), Dirr and Heuser (1987), Young & Young (1992), Bujarska-Borkowska (2002-2006), Gokturk et al., (2017). Seeds were immersed in an ash solution for 48 and 144 hours based on the recommendations of Gokturk and Yilmaz (2015). Seeds were subjected to the following pretreatments (Table 1).

Table 1. Pretreatments

Pretreatment	Duration
Immersing in C ₂ H ₆ O	48 and 144 hours
Immersing in H ₂ O ₂	1, 3 and 6 hours
Immersing in ash solution	48 and 144 hours
Scarification in H ₂ SO ₄	1, 3 and 6 hours
Scarification in H ₂ SO ₄ followed by immersing in ash solution	1, 3 and 6 hours + 48 and 144 hours
Mechanical scarification	-
Control (No treatment)	-

The ash solution was prepared by mixing 50 g of wood ash and 1 liter of water. While the seeds were pretreated with the ash solution, the solution was renovated every two days to allow good aeration of the seeds. For the mechanical scarification process, 100 seeds were clamped at the seed tip and then sandpapered.

Germination Experiments

International standards for germination testing require testing of 400 seeds (ISTA 1993). However, ISTA seed testing rules allow for testing of not less than 100 seeds for special circumstances, such as high seed costs and difficulty in attenuation of seeds (Milivojevic et al., 2018). Due to the difficulty in attenuation of seeds in this study, 100 seeds were used for each treatment. The experimental design was a randomized complete block with four replications (i.e., twenty-five seeds in each replication). In order to eliminate the need for warm stratification, seeds were sown in pots in a greenhouse (22-25°C, 60% humidity) in December 2016 and pots were transported to an open area approximately three months following (March 2017) the seed sowings. A 3:1 mixture of peat and perlite was used for the germination media. The study regarded the criterion of germination to be the emergence of cotyledons on to the surface of the mixture. Germinations occurred in the second spring. Germinated seeds were counted periodically

twice a week after germinations had started until the germinations ceased.

Statistical Analysis

Germination percentage (GP) of seeds was determined separately for each pretreatment and pretreatment group for both of the two species.. The following formula (Ahmadloo et al., 2014) was used to determining GP,

$$GP = \frac{\sum n}{N} \times 100 \quad (2)$$

where n is number of germinated seeds and N is total number of sown seeds.

GP data was transformed using arcsine square root (\sqrt{P}) (Compton, 1994) to normalize error distribution. The mean GP was used to determine the differences between species. The differences between seed properties of common hawthorn and azarole seeds were analyzed by using two independent sample T-tests using SPSS (Version 19, 2019) statistical package program. ANOVA and Duncan's multiple range tests were used to compare treatments and treatment groups in determining whether they showed any statistically significant differences at a significance level (α) of 0.05.

Results and Discussion

Results of the T-test showed that differences between the seed diameter, seed weight and GP of common hawthorn and azarole seeds were statistically significant ($p < 0.05$) (Table 2).

Table 2. Results of the T-test (two independent samples) of seed properties

Seed Properties	F	t	Sig.	Common hawthorn				Azarole			
				Mean	Std.	Min.	Max.	Mean	Std.	Min.	Max.
					Error				Mean		
Diameter (mm)	15.8	-7.02	0	5.96	0.06	4.94	8.94	6.69	0.09	4.81	8.96
Length (mm)	1.26	0.72	0.47	8.29	0.08	6.28	10.64	8.22	0.07	6.58	9.98
Thinnest coat (mm)	1.54	1.86	0.07	1.4	0.05	0.85	4.61	1.3	0.03	0.83	1.82
Thickest coat (mm)	5.84	-7.49	0	2.39	0.03	1.82	3.08	3.07	0.08	2.07	7.77
Seed weight (g/100 seeds)	0.37	-7.41	0	13.62	0.37	11.08	14.31	16.77	0.21	15.93	17.58
Moisture content (%)	1.3	1.78	0.13	8.06	0.05	7.94	8.19	7.96	0.02	7.91	7.99
GP (%)	1.96	-6.28	0	16.53	2.08	0	64.35	37.31	2.57	0	88.61

Seed diameter, seed weight and GP of the azarole seeds were greater than the common hawthorn seeds. Seed coat thicknesses from the thinnest and thickest part of the seed were also statistically different ($p < 0.05$) between azarole and common hawthorn seeds. Azarole seeds had both the thinnest and thickest seed coats. This result shows that azarole seeds have the most variability of seed coat thicknesses, even within the same seed type. These interspecific differences in coat thickness found in this study are similar to those reported by St. John (1982) and Gokturk et al., (2017).

In hawthorn species, it is stated that the number of seeds in 1 kg varies between 9500 and 20000 (Piotto et al. 2003). The reason for the wide range of seed numbers in a kilogram is due to the high variability of seed size

among the species (St. John, 1982; Gokturk et al., 2017). Azarole seeds are larger than the seeds of some other hawthorn species (Gokturk et al. (2017). In this study, it was revealed that the number of seeds in one kilogram varied between 6988-8475 based on the result that 100 seed weights varied between 11.8 and 14.31 (Table 2).

Treatments applied to common hawthorn seeds did not show a statistical difference ($p > 0.05$) in GP versus the control. Germination did not occur in seeds treated with ethanol, while the GP of seeds treated with other treatments achieved between 5.04% and 31.40% (Table 3). GP achieved from pretreatment groups varied between 12.07% and 23.98% and were not statistically different ($p > 0.05$) from the control seed groups (21.05%) (Figure 3).

Table 3. GP of common hawthorn (F: 0.864) and azarole (F: 7.27) seeds according to pretreatments

Pretreatment	N	Common Hawthorn		Azarole	
		GP (%)	Std. Error	GP (%)*	Std. Error
144h- C ₂ H ₆ O	4	0.00	0.00	0.00 e	0.00
48h- C ₂ H ₆ O	4	0.00	0.00	0.00 e	0.00
6h-H ₂ O ₂	4	7.17	7.17	0.00 e	0.00
3h- H ₂ O ₂	4	10.07	5.81	13.88 de	8.59
3h-H ₂ SO ₄ + 144h-ash solution	4	25.93	9.60	33.23 cd	11.56
Control	4	21.05	7.68	34.77 cd	4.17
Mechanical scarification	4	14.34	8.28	35.29 cd	6.84
6h- H ₂ SO ₄	4	16.09	16.09	37.26 bc	14.86
3h- H ₂ SO ₄ + 48h-ash solution	4	22.78	13.80	37.43 bc	5.45
1h- H ₂ SO ₄	4	18.98	13.15	41.93 abc	11.57
48h- ash solution	4	17.84	12.09	43.89 abc	6.99
3h- H ₂ SO ₄	4	5.04	5.04	44.31 abc	5.88
1h- H ₂ SO ₄ + 144h-ash solution	4	12.21	7.26	44.66 abc	4.33
1h- H ₂ SO ₄ + 48h-ash solution	4	27.14	10.49	51.76 abc	5.56
6h- H ₂ SO ₄ + 48h-ash solution	4	31.40	11.84	55.52 abc	4.32

Table 3. (Continued)

Pretreatment	N	Common Hawthorn		Azarole	
		GP (%)	Std. Error	GP (%)*	Std. Error
1h- H ₂ SO ₄	4	18.91	7.25	57.67 abc	5.07
144h-ash solution	4	17.84	12.09	60.60 ab	6.76
6h- H ₂ SO ₄ +144h-ash solution	4	24.41	2.47	64.98 a	8.34

*significantly different at $\alpha=0.05$

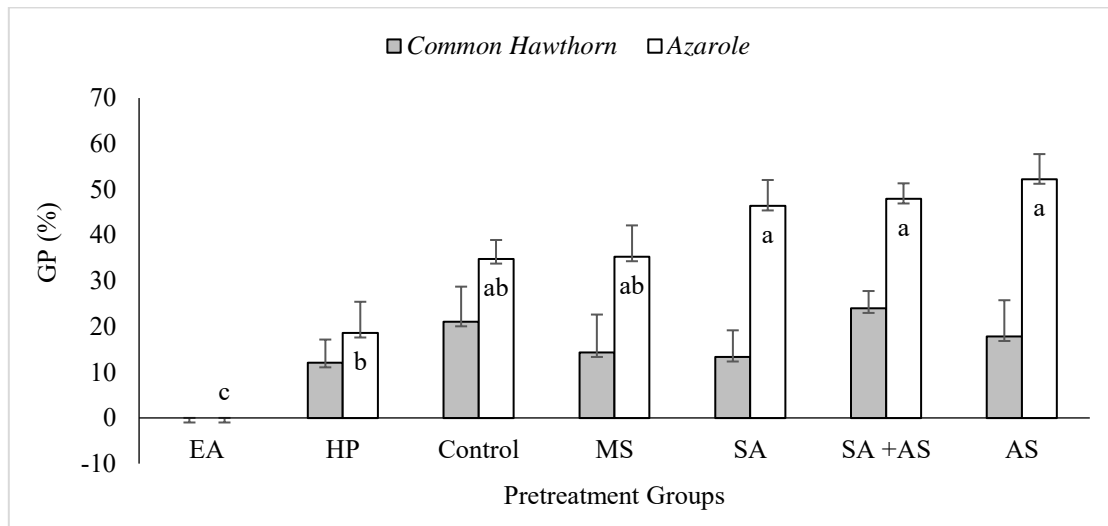


Figure 3. GP of common hawthorn (F: 1.80) and azarole (F: 10.65) seeds according to the pretreatment groups (EA: C₂H₆O , HP: H₂O₂, MS: Mechanical scarification, SA: H₂SO₄, SA+AS: H₂SO₄+ash solution, AS: Ash solution).

The GP of azarole seeds which were subjected to the ash solution was significantly ($p<0.05$) higher compared to control treatments. The greatest germinations (64.98%) occurred from seeds subjected to sulfuric acid for 6 hours followed by soaking in ash solution for 48 hours (Table 3), while the greatest GP (52.24%) in the pretreatment groups occurred in the ash solution pretreatment group (Figure 3). However, the GP of H₂SO₄ and H₂SO₄ + ash solution pretreatment groups were not statistically different from the ash solution pretreatment. Hou & Simpson (1994) stated that breaking dormancy by alkaline treatments is related to removing the barrier to water uptake formed by the seed coat. In another study conducted by Gokturk & Yilmaz (2015), it was found that an alkaline ash solution pretreatment increased the GP of *C. orientalis* seeds. This result shows that the alkaline ash solution pretreatment can also be used to break

germination barriers in the seeds coats of other *Crataegus* species.

Azarole seeds subjected to H₂O₂ treatments germinated at lower rates. Increasing the duration of scarification in H₂SO₄ and the H₂O₂ treatments caused decreases in GP. While the decrease in GP of seeds treated with H₂SO₄ was not statistically significant ($p>0.05$), the decrease in GP of seeds treated with H₂O₂ was found to be statistically significant ($p<0.05$). Increasing the duration of H₂O₂ treatments caused statistically significant decreases in the GP of azarole seeds. H₂O₂ is a weak acid and generally used for seed disinfection. It causes decomposition on the surface of the seed coat but not as much as H₂SO₄. Therefore, treated seeds such as wheat and legume seeds for a period of a day with diluted H₂O₂ solutions improve germination rates (Dhillon, 1961). However, in this study, H₂O₂ treatments did not improve germination of common hawthorn and azarole seeds.

Germination did not occur in azarole seeds treated with ethanol as had been seen with common hawthorn seeds. Dhillon (1961) stated that if seeds first soaked in C_2H_6O for an hour and then placed in water, imbibition of water can be achieved. C_2H_6O enters the openings on seed coats and furnishes a path for the inward diffusion of the water if the water absorbing tissues were present on the seed surface. In addition, C_2H_6O is able to remove inhibitors from seed coats (Kim et al., 2016). Therefore, C_2H_6O pre-treatments have been reported to be effective in the breakage of dormancy (Idu & Omonhinmin, 2002). The absence of germination may be due to the excessive holding time according to Dhillon (1961)'s recommendation. Another reason may be the concentration of C_2H_6O . Salehi et al., (2008) found that an application of a high concentration of C_2H_6O has a lethal effect on the seeds of embryos. Therefore, results showed that both the high concentration of C_2H_6O and the long application period has a possible fatal effect on seeds of both *Crataegus* species, despite the seeds having a hard and thick coat.

The differences between the GP of azarole seeds in pretreatment groups were statistically significant ($p < 0.05$). The highest GP was obtained from soaking both in H_2SO_4 and H_2SO_4 + ash solution pretreatments returning 44.49%, 46.41%, 47.93% and 52.24%, respectively. GP of seeds subjected to mechanical scarification was 35.29% and it was not statistically different from the GP (34.77%) obtained from control seeds (Figure 3).

Scarification pretreatments are suggested to break seed dormancy of hawthorn seeds that is caused from seeds having thicker seed coats or being less permeable (Lasseigne & Blazich, 2008). In this study, the mechanical scarification pretreatment provided greater germination of azarole seeds compared to C_2H_6O and H_2O_2 pretreatments. However, the GP of azarole seeds which were subjected to sulfuric acid was significantly ($p < 0.05$) higher as compared to mechanical scarification.

It is possible that different responses could occur among seeds of different *Crataegus* species subjected to similar

pretreatments because of differing seed dormancy types and degrees. Therefore, the pretreatments that are suggested to break seed dormancy of *Crataegus* species differ (Hartman et al., 1997). As in the present study, applying similar pretreatments to seeds of different species may be useful for differentiating the degree of dormancy among species. The results of this study indicate that the degree of dormancy of common hawthorn seeds was higher than the seeds of azarole (Figure 3). Persson et al., (2006) found that the mechanical resistance to the splitting of endocarps decreased during the warm stratification period, thus the GP of common hawthorn seeds increased when increasing the duration of warm stratification. However, the stratification pretreatment was not applied in this study. To eliminate the need for warm stratification, sowings were carried out in the greenhouse. Hartmann et al., (1997) specified that the stratification process is actualized in natural conditions if sowings are done immediately after the seeds ripen and it is not necessary to do extra stratification pretreatments. However, results showed that sowing in December at greenhouse conditions was not enough for the stratification needs of the seeds used in this study. In support of this result, referring to Tyskiewicz (1949), Bujarska-Borkowska (2002) which stated that even if the seeds of *Crataegus* species are sown immediately following ripening or exposure to acids, seedling emergence occurs only in the second spring. Similarly, Gultekin et al., (2006) indicated that the seeds of *Crataegus* species germinated at the beginning of the second spring after experiencing cold then warm conditions in nature, even though the seeds had ripened.

Germination is considered as complete when the embryo applies force to its surrounding restrictive tissue. The properties of the tissue covering the embryo and the outer seed coat are decisive in this process. The removal of tissues such as endosperm and pericarp surrounding the embryo is enough to complete germination in seed coat dormancy (Ahmadloo et al., 2017). However, the removal of the seed coat does not provide normal development of embryos. Therefore, embryo dormancy is more effective than seed

coat dormancy (Bewley et al., 1994). In this study, from the viewpoint of these statements, germinations that occurred in the second spring could be caused by stratification needs of the seeds that were not resolved.

Conclusion

In conclusion, the current study showed that azarole seeds have greater seed diameter and seed weight compared to common hawthorn seeds. Moreover, the fact that the thinnest and thickest seed coat thicknesses belong to the azarole seeds shows that azarole seeds have the most variability in seed coat thickness, even within the same seed type. Pretreatments did not affect the germination of common hawthorn seeds. However, ash solution treatments increased GP of the azarole seeds and it was found to be more effective compared to mechanical scarification, hydrogen peroxide and ethanol treatments. The mechanical scarification treatment was less effective than scarification in sulfuric acid for breaking seed dormancy of azarole seeds. In order to break seed dormancy of azarole seeds, treating seeds with ash solution can be carried out in combination with chemical scarification in sulfuric acid. It may be useful to determine pretreatments by applying a combination of cold or warm stratification with ash solution treatments enabling high rates of germination.

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