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Seed characteristics of *Amygdalus arabica* in Adıyaman region of Turkey

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Abstract: Mountain almond (*Amygdalus arabica*) is a shrub with naturally distributing in Turkey, Iran, Iraq, Syria, Jordan and Saudi Arabia. *A. arabica* also known as bitter almond and can reach up to 2.5 m. The natural distribution of mountain almond is generally in the Southeast Region of Turkey. The present study carried out to determine the morphological and physiological characteristics of the seed of mountain almond. The seeds were collected from three different populations of *A. arabica* in Adıyaman. For different each populations seed weight, height, width, thickness, and 1000-seed weight were measured in the laboratory. Morphological characteristics of *A. arabica* seeds varied according to their populations. Seed germination tests were conducted at 20 °C, after prechilling for 0, 3, 6, and 9 weeks at 4 °C. Prechilling treatments of 3 weeks were insufficient, but 6 and 9 weeks of prechilling were found to eliminate seed dormancy and led to the highest germination percentage. The average germination percentage after 0, 3, 6, and 9 weeks of prechilling were 3.1%, 49.6%, 80.4%, and 81.1%, respectively. The average mean germination time was approximately between 8 and 10 days.

Keywords: mountain almond tree, hot-arid areas, seed germination, prechilling, dormancy

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Introduction

Mountain almond (*Amygdalus arabica* Olivier) is a shrub with naturally distributing in Turkey, Iran, Iraq, Syria, Jordan and Saudi Arabia (Fig. 1). *A. arabica* can reach up to 2.5 m. and also known as Bitter almond (Yaltırık, 1971; Kester & Asay, 1975; Kester et al., 1991; Browicz & Zohary, 1996). The natural distribution of mountain almond is generally in the Southeast Region of Turkey. The under and upper surface of 10–40 mm length and 3–5 mm thick leaves are felt-like feathered as a sign of drought resistance. Fruits are spherical or egg-shaped (ellipsoid) about 2.5 cm long. The *A. arabica* generally has ellipsoid (*A. arabica* var. *spartioides*) shaped fruit structure in Turkey (Davis, 1972; Browicz & Zohary, 1996). Its distribution in the region is generally between 500–1650 m (Demirbağ et al., 2018). It is frequently seen in areas eroded, on dry stony slopes, in forested areas and steppe environments and on road slopes in the region. Fruits are edible while fresh, almond (seed) is not eaten because of bitter taste (Meikle, 1966; Yaltırık, 1971; Browicz & Zieliński, 1984). Flowering takes place in March–April (Davis, 1972; Yıldız, 2013). The flowers are individual, stemless and appear before leafing. It is a broom-like shrub (Miller & Cope, 1996).

This species, which is found in poor regions in terms of woody species, also supports the wildlife in the region. It is used as firewood in places where there is a limited burning material. *A. arabica* is a species that can sustain its existence even on poor

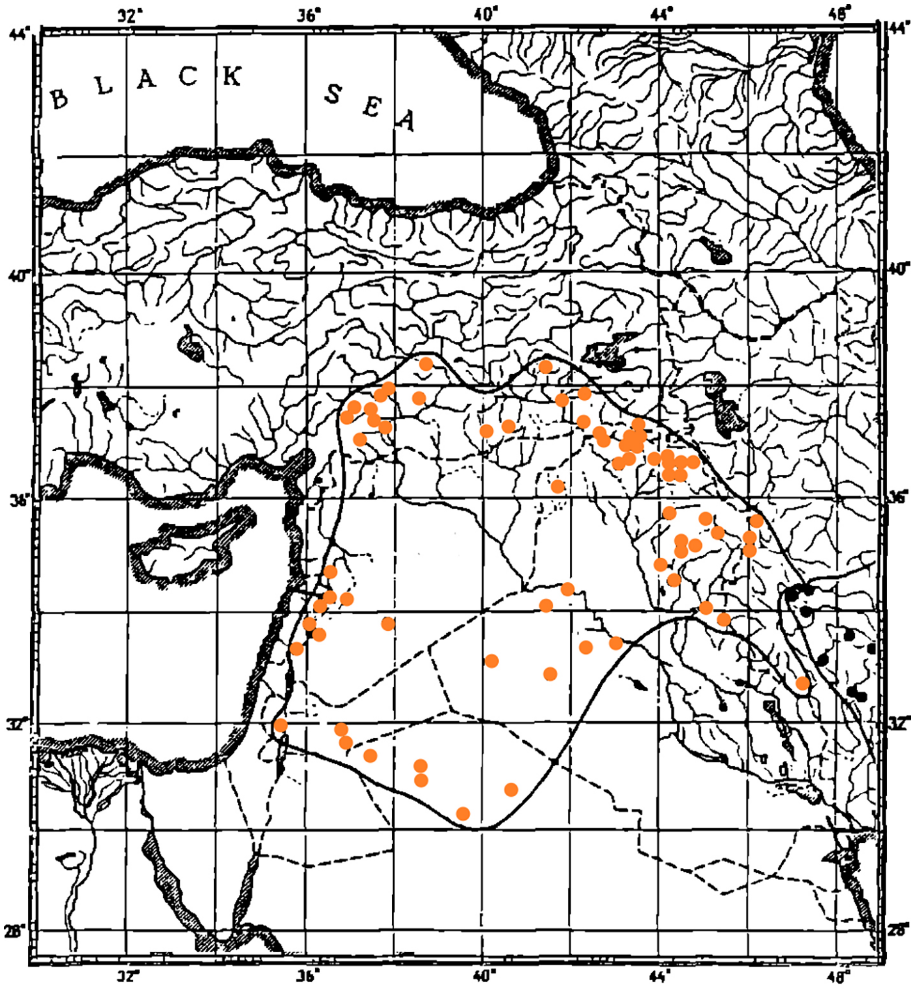

Fig. 1. The distribution of *A. arabica* in Turkey, Iran, Iraq, Syria, Jordan and Saudi Arabia (Browicz & Zohary, 1996)

soil. The roots of shrub reach into deep and hold soil even in the riskiest areas of erosion. The species is a very valuable native plant material for the plantation of stony, rocky fields and the slopes with the risk of erosion especially around natural distribution areas (Yaltırık, 1971; Browicz & Zieliński, 1984; Grasselly, 1990).

The studies on mountain almonds have been increasing in recent years due to its usefulness for different purposes in difficult places (Gholami et al., 2010; Hosenzadeh & Saeb, 2011; Bayazit et al., 2011; Rahemi et al., 2009; Yıldız, 2013; Goujani et al., 2013; Köse et al., 2015; Akgün et al., 2018, Kayra et al., 2018). *A. arabica* is very useful plant material for the hot-arid regions due to its resistance to extreme drought conditions. The suitable populations in high altitudes are also available to be used in the planting of cold-arid areas.

There have been several studies on the germination of almond species seeds using different techniques and methods. (Khalil & Al-Eisawi, 1998; Garcia-Gusano et al., 2004; Rouhi et al., 2005a; Rouhi et al., 2005b; Rahemi et al., 2009; Gholami et al., 2010; Hosenzadeh & Saeb 2011; Kayra et al., 2018). *Amygdalus arabica* seed germination has been studied with bioregulators in a few studies (Khalil & Al-Eisawi, 1998; Rahemi et al., 2009) but neither extensively studied on the morphological nor physiological characteristics of its seeds.

In this study, physiological and morphological (weight, length, width and thickness) characteristics of mountain almond seeds collected from three different populations in Adıyaman region of Turkey were investigated. The dormancy and depth of dormancy in *A. arabica* seeds were examined. In addition, it was hypothized that there are degrees of variations between populations in terms of seed morphological and physiological characteristics.

Materials and Methods

The fully mature fruits were collected from three populations of *Amygdalus arabica* in southern Turkey in July, 2019 (Table 1; Fig. 2). The collected fruits were packed in plastic bags and stored under laboratory conditions.

Mountain almond fruits were dried at room temperature in the laboratory and the seeds are extracted from their husks (mesocarp). The seeds dried for about 1 week until their moisture content was reduced to 8% and then stored in a refrigerator within bottles.

Measurement of morphological characteristics of seeds

Morphological measurements were performed on 100 seeds randomly selected from each population. Length, width, thickness, and weight were measured in each seed. Measurements were carried out with 0.0000 g and 0.00 mm sensitivity. The endocarp thickness and embryo/seed ratio (ESR) were determined. Also, according to the rules of ISTA (2019), 1000 seed weights from 800 (8×100) seeds were calculated.

Dormancy level and pretreatments

In order to determine the dormancy level and prechilling time of the species, the seeds collected from three different populations (Table 1) were prechilled at 4 °C in incubators for 0 (Control), 3, 6, and 9 weeks.

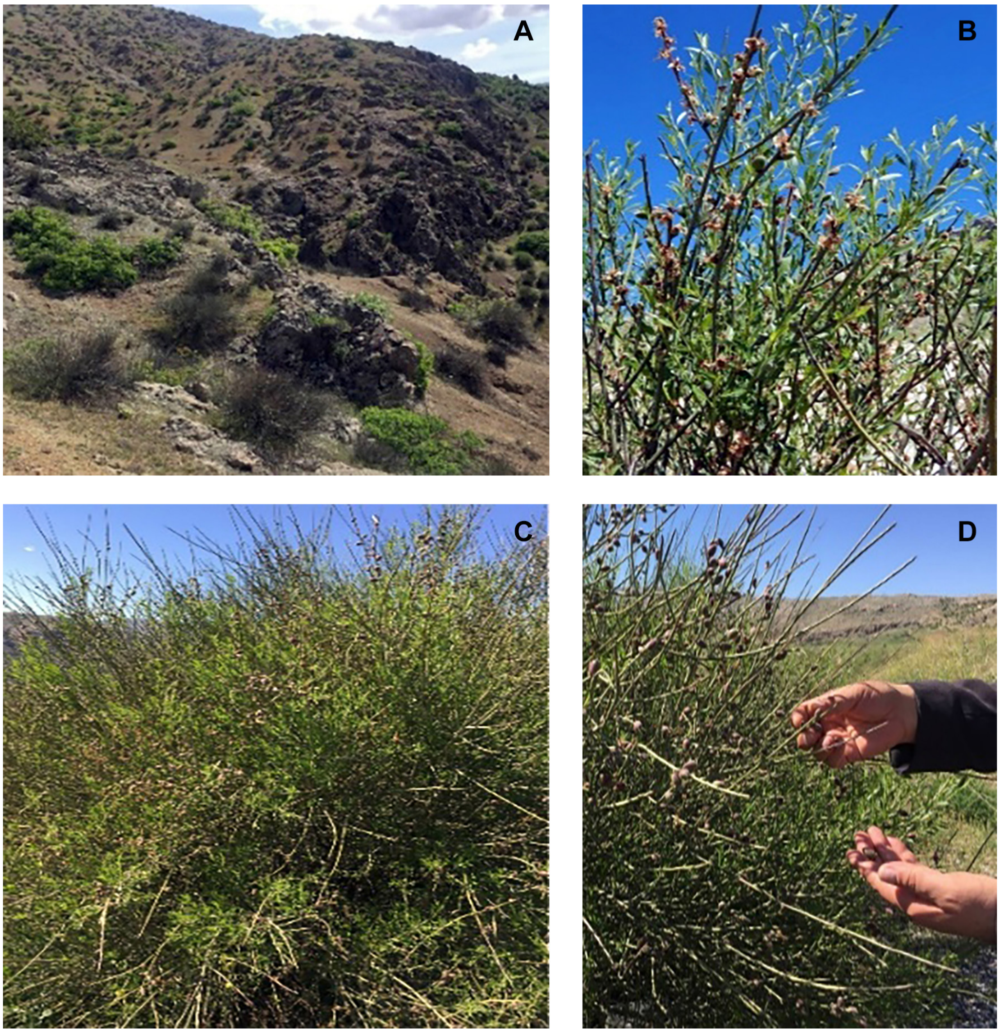

Fig. 2. Natural distribution sites of *A. arabica* (A), flower and fruit (B, C) and the collection of mature fruit (D)

Table 1. Information on the collected seed materials

Populations	Seed collection date	Latitude	Longitude	Altitude [m]	Aspect
Akyazı	July 18, 2019	37°30'	38°17'	580	South
Çemberlitaş	July 20, 2019	37°48'	38°18'	770	South
Yassıkaya	July 23, 2019	37°55'	38°36'	630	South

During prechilling (4 °C), distilled water was added with spray when necessary to prevent moisture deficiency in Petri dishes with a double-layer filter paper on which seeds were placed.

Germination test

Germination tests were performed in glass Petri dishes (12 cm diameter) in incubators at 20 °C with 150 seeds (3 dishes × 50 seeds) on double-layer filter paper. As a result of the preliminary tests, the suitable germination temperature for the seeds of the species was determined as 20 °C. Before the germination test, the seeds were kept in 10% sodium hypochlorite (NaClO) solution for 10 minutes in order to prevent fungal and bacterial infections. After surface sterilization, the seeds were washed three times with sterile distilled water (Cantos et al., 1998; Işıkalan et al., 2008). Filter papers were replaced when a fungal infection occurred. The seeds were considered germinated when the seed radicles were about 3 mm and geotropism was observed. Once every two days, Petri dishes were checked. The germinated seeds were recorded and removed. Germination tests were terminated after 28 days.

Germination parameters

The germination percentage (GP) and mean germination time (MGT) were calculated by the following formulas during germination tests (Bewley et al., 2013):

$$GP(\%) = \frac{\sum n_i}{N} \times 100$$

- GP (%): Germination percentage,
- n_i: The number of germinated seeds at day i,
- N: The total number of incubated seeds per test.

$$MGT = \frac{\sum (t_i \times n_i)}{\sum n_i}$$

- MGT: The mean germination time,
- t_i: The number of days since the beginning of the test,
- n_i : The number of germinated seeds recorded for day t_i.

Statistical analyses

The data of seed weight, length, width, thickness, 1000-seed weight, germination percentage and mean germination time were evaluated by analyses of variance. One-Way ANOVA test was performed to reveal the interaction of prechilling and population factors in all of the data obtained after the germination test. GP values were transformed using arcsine formula to normalize error distribution prior to variance analyses. Differences among groups were determined using Duncan's multiple range test when a significant effect was identified.

Results

Morphological characteristics of the seeds

In terms of morphological features, *A. arabica* seeds have been determined to significant differences (Table 2, Fig. 4). The average seed length, width, thickness, and weight were 14.99 mm, 10.57 mm, 7.89 mm, and 0.546 g respectively. In terms of seed weight from the populations were determined respectively as the heaviest to lightest; Çemberlitaş, Akyazı, and Yassıkaya. The average 1000-seed weight at about 8% MC for the three populations was 487.6 grams.

Also, the average of embryo/seed ratio, endocarp weight, embryo weight and endocarp thickness were 36.65%, 0.354 mg, 0.203 mg, 1.07 mm respectively (Table 3). The seed in the tables and texts is the material extracted from the fruit with endocarp and embryo inside. The endocarp, a hard shell, is the hard structure that protects the embryo. In this study, the embryo is the entire part under the endocarp. For practical purposes, the weight of the seed coat was included in the embryo weight (Fig. 3).

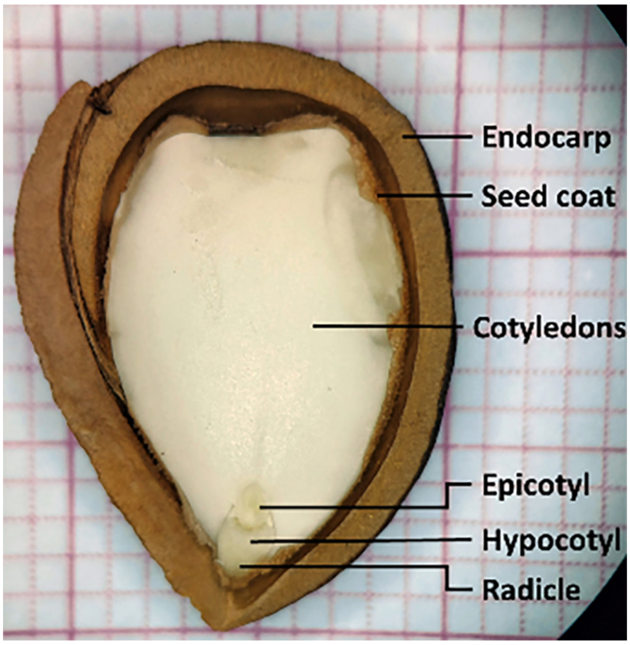

Fig. 3. Section through an *A. arabica* seed with a visible embryo inside

Table 2. Morphological characteristics of the *A. arabica* seeds

Populations	Length [mm]	Width [mm]	Thickness [mm]	Weight [g]	1000-seed weight [g]
Akyazı	15.34 a	10.39 a	7.66 c	0.542 ab	487.6
Çemberlitaş	14.75 b	10.68 a	7.91 b	0.573 a	498.8
Yassıkaya	14.87 ab	10.65 a	8.12 a	0.523 b	494.2
Average	14.99	10.57	7.89	0.546	487.6

[1]The values on the same column followed by the same small letters are not significantly different at P<0.05.

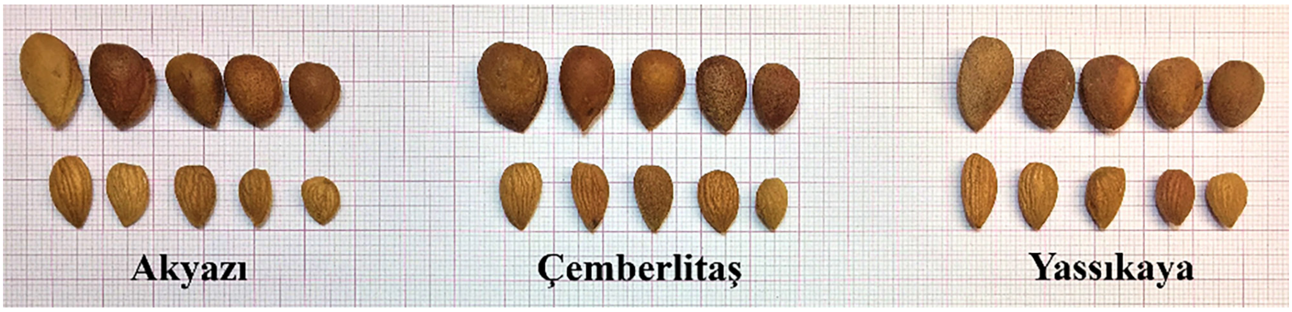

Fig. 4. Different size of seeds (upper) and embryos (bottom) from three different populations

Dormancy level and prechilling requirements

The germination percentage and mean germination times of *A. arabica* seeds were significantly affected by both the prechilling and population. The prechilling × population interaction effect was also significant on GP and MGT (P<0.01) (Table 4).

Significant differences were determined between the prechilling treatments in terms of the germination percentage of seeds germinated at 20 °C. Prechilling treatments of 3 w were insufficient, but 6 and 9 w of prechilling were found to eliminate seed dormancy and led to the highest GP. The average germination rates after 0 (Control), 3, 6, and 9 weeks of prechilling were 3.1%, 49.6%, 80.4%, and 81.1%, respectively (Table 5, Fig. 5).

Table 3. Seed, endocarp and embryo weights and embryo/seed ratio values of the populations

Populations	Seed weight [g]	Endocarp weight [g]	Embryo [g]	Embryo/Seed Ratio [%]	Endocarp thickness [mm]
Akyazı	0.550 a	0.352 a	0.198 ab	36.22 ab	1.24 a
Çemberlitaş	0.555 a	0.337 a	0.218 a	39.24 a	0.88 c
Yassıkaya	0.567 a	0.372 a	0.194 b	34.49 b	1.10 b
Average	0.557	0.354	0.203	36.65	1.07

[1]The values on the same column followed by the same small letters are not significantly different at P<0.05.

Table 4. Effects of the prechilling and population on germination percentage (GP) and mean germination time (MGT) of *A. arabica*

Source	GP				MGT		
	df	MS	F	p-value	MS	F	p-value
Prechilling (A)	3	6019.2	544.4	0.000	508.17	251.93	0.000
Population (B)	2	93.4	8.4	0.002	22.03	10.92	0.000
A × B	6	125.6	11.3	0.000	7.85	3.89	0.007
Error	24	11.1			2.02		

Table 5. Germination percentage for *A. arabica* seeds at 20 °C after 4 prechilling regimes

Populations	Prechilling duration				Average
	0 (control)	3 weeks	6 weeks	9 weeks	
Akyazı	2.7 c[1]	42.7 b	82.0 a	80.7 a	52.0 A[3]
Çemberlitaş	4.0 c	60.7 b	69.3 a	71.3 a	51.3 A
Yassıkaya	2.7 c	45.3 b	90.0 a	91.3 a	57.3 A
Average	3.1 C[2]	49.6 B	80.4 A	81.1 A	53.6

[1]The values on the same row followed by the same small letters are not significantly different at p<0.05.
[2]The values on the same row followed by the same capital letters are not significantly different at p<0.05.
[3]The values on the same column followed by the same capital letters are not significantly different at p<0.05.

Table 6. Mean germination times (day) of *A. arabica* seeds after different prechilling regimes

Populations	Prechilling duration				Average
	0 (control)	3 weeks	6 weeks	9 weeks	
Akyazı	23.7 c[1]	8.7 b	4.4 a	4.5 a	10.3 A[3]
Çemberlitaş	16.6 c	5.9 b	4.0 a	4.2 a	7.7 A
Yassıkaya	19.3 c	7.4 b	3.7 a	3.6 a	8.5 A
Average	19.9 C[2]	7.4 B	4.0 A	4.1 A	8.8

[1]The values on the same row followed by the same small letters are not significantly different at $p<0.05$.
[2]The values on the same row followed by the same capital letters are not significantly different at $p<0.05$.
[3]The values on the same column followed by the same capital letters are not significantly different at $p<0.05$.

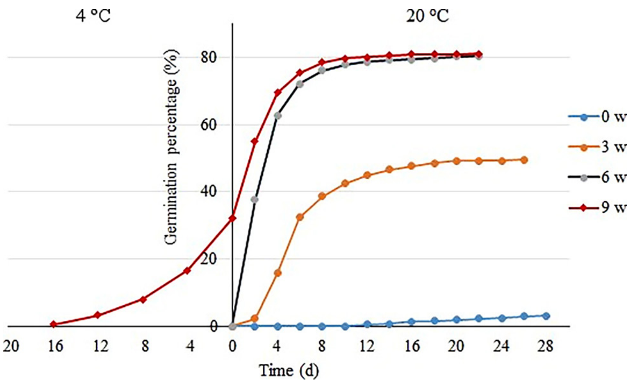

Fig. 5. Average germination percentage of *A. arabica* seeds (w – week, d – day)

The germination percentage was very low in the 0 w (control) without prechilling. About half of the seeds germinated with 3 w prechilling duration. Following the 6-w and 9-w prechilling durations have almost similar germination percentage. In addition, seeds started to germinate at 4 °C in the late of 9 w prechilling duration.

The prechilling duration also affected the time required to germinate. In general, longer prechilling time eliminates the seed dormancy more easily and led to earlier germination. The average MGT was approximately between 8 and 10 days. There was no statistically important differences for MGT between populations. (Table 6).

Discussion

This study of seed characteristics of *A. arabica* revealed the morphological seed characteristics, the depth of dormancy and the effects of prechilling duration on the germination behavior. Although germination tests terminated after 28 days, experiments showed that the three-week test period was sufficient germination time for *A. arabica* seeds.

Significant differences were identified in the three populations examined according to mountain almond seed morphological characteristics (Table 2). The seed length, weights and thicknesses varied between populations. This result is consistent with the hypothesis that one of the factors affecting seed morphology is the habitat variation of a species. (Fenner & Thompson, 2005).

A. arabica fruit are generally smaller than most of other *Amygdalus* species. According to the average fruit length-width, *A. arabica* (15–10 mm) is smaller than *A. lycioides* (17–9 mm), *A. orientalis* (25–16 mm) and *A. communis* (46–27 mm) (Karataş, 2007; Köse, 2014). The result of the morphological measurements of the three populations of A. arabica seeds, the average length and width were determined as 14.99 mm and 10.57 mm, respectively. The average 1000-seed weight of *A. arabica* for the three populations (487.6 g) was roughly similar to the previously recorded average 1000-seed weight of the species (449.4 g) (RBG, 2020).

In the ecology of a plant species, seed dimensions are vital for maintaining viability. The main determinant of seed dimension and shape are genetic characteristics and environmental factors (Leishman et al., 2000; Flores, 2002; Desai, 2004). The seed mass is also highly dependent on environmental conditions during seed development (Michaels et al., 1988; Castro et al., 2006). In this study, in terms of seed dimension, significant variations were determined within and among the populations. Similar studies have been reported in many other studies in the literature and almond species including *Amygdalus arabica* Oliv., *Amygdalus orientalis* Mill., and *Amygdalus turcomonica* Lincz. (Köse et al., 2015), *Amygdalus communis* L. (Talhouk et al., 2000), *A. orientalis* Mill. (Shalaby et al., 1997), *A. orientalis* Mill. and *A. turcomenica Lincz.* (Ak et al., 2001), *A. orientalis* Mill. and *A. turcomenica* Lincz. (Bayazıt, 2007).

Generally, the depth of dormancy varies depending on the species, the habitat of the species or individuals of the same locality (Bewley & Black, 1994; Copeland & McDonald, 1999; Schmidt, 2000; Bewley et al., 2013). In the prechilling period, 3-week prechilling was insufficient to eliminate the presence of dormancy of *A. arabica* seeds. However, 6 to 9 weeks prechilling showed full germination potential in the seeds. After 6 weeks prechilling, the seeds obtained from three populations had similar dormancy of depth and germination rates. Similarly, prechilling between 45 or 60 days has been determined to be sufficient on the germination of *A. arabica* in a study by Khalil and Al-Eisawi (1998). The experiments suggest that 6 weeks of prechilling is sufficient for *A. arabica* seeds to break the dormancy.

Generally, warm incubation is used for seeds that have morphological dormancy. (Baskin & Baskin, 2004). This study showed that having only a physiological dormancy of mature *A. arabica* seeds (ripen around July) that requires about 6 weeks of prechilling for germination. Although mountain almond has a thick endocarp (Table 3), it has been detected that there is no physical dormancy due to the easy entry of moisture and air from the endocarp.

The distribution range of the species is closely related to the depth of seed dormancy (Schmidt, 2000; Baskin & Baskin, 2001; Fenner & Thompson, 2005). In this study, the seeds from the three populations demonstrated a similar depth of dormancy, likely because of their relatively close distribution in a limited area of the southern of Turkey.

The germination temperature, very effective factor on seed germination parameters, is highly related to the ecology and the distribution range of the species (Fenner & Thompson, 2005; Yılmaz, 2010). The preliminary tests and the study demonstrated that germination test at 20 °C is a suitable temperature for the seed germination of *A. arabica,* mainly distributed in hot-arid areas. On the other hand, the seeds of many plants cannot germinate at low temperature such as 4 °C. Khalil and Al-Eisawi (1998), obtained significantly highest germination percentage (63–67%) on *A. arabica* seeds after prechilling at 45–60 days at 5 °C. In this study, the seeds were started to germinate at 4 °C in the late of 9 w prechilling duration after the complete elimination of dormancy. This indicates that *A. arabica* can adapt to the relatively cold areas.

This study also showed that *A. arabica* seeds have a deep physiological dormancy. Also, the seed sowing time is critical in the forest nursery in Turkey. The dormancy of seeds naturally eliminates during the winter. For seed propagation, the dormant seeds of *A. arabica* should be sown around the beginning of January. On the other hand, nondormant seeds could also be sown in the spring after approximately 6 weeks of prechilling. *A. arabica* seeds are dry stored (orthodox) seeds (RBG, 2020). Dry seeds of the species can easily be stored in seed banks for the long period (Yılmaz et al., 2014; Yılmaz & Ok, 2015).

The species is a very valuable native plant in hot-arid regions with extreme growing conditions. This study demonstrated that the seeds need about 6-week chilling pretreatment and are not difficult to germinate. *A. arabica* should be used in the plantation of stony, rocky, and the slopes with the risk of erosion especially around natural distribution areas. The suitable populations at high altitudes can also be one of the best seed origins in the planting of cold-arid areas. In addition, a couple of dense populations of this neglected species should be reserved as a genetic conservation sites.

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