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# The effect of seed pre-soaking, burial depth and site conditions on the survival and growth of wild almond, *Amygdalus scoparia*

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## ABSTRACT

Wild almond (*Amygdalus scoparia*) is one of the most important species that provides a variety of ecological functions in the Zagros forest ecosystem. In this study, we investigated the effects of some seed treatments on the survival and growth of *Amygdalus scoparia* after seed planting at different aspects and elevations under natural conditions. The seed pretreatments consisted in soaking them in water at cold (10 °C) and warm (80 °C) temperatures for 24 hours. The impact of these pretreatments was evaluated on the survival rate (%), height and diameter growth of newly emerged seedlings. Based on Duncan's test, the height of seedlings established on the north-facing aspect was significantly higher than the other aspects, while the lowest seedling height was observed in the south-facing aspect. The height of seedlings established on east- and west-facing aspects had intermediate values and were statistically similar. The tallest seedlings were observed when seeds were buried at a depth of 2 cm and seedling height tended to decrease with increasing burial depth. Seedlings established on the north-facing aspect at both elevation levels (1200 and 1800 m) were significantly taller than those found on the south-facing aspect. Overall, the use of cold water as a seed pre-treatment in conjunction with a north-facing seedling at a burial depth of 2 cm maximize the survival and the development of *Amygdalus scoparia* seedlings.

**Key words:** Wild almond, Afforestation, Seed, Burial depth, Soaking, Physiographic conditions, Zagros.

## INTRODUCTION

Zagros forests cover over one-fifth of the Iran territory and are classified as a semi-arid domain (Sagheb-Talebi *et al.* 2004; Heydari & Atar Roushan 2011). The vital role of these semi-arid forests in sustainable development is underlined by the significant utilization of non-wood products for the people livelihood (Mahdavi *et al.* 2011). Restoration and afforestation of these lands are problematic because of the high atmospheric evaporative, particularly during the growing seasons, the irregular precipitation distribution, the forest overharvesting and overgrazing as well as landclearance (Pourmoghadam *et al.* 2013). Zagros forests are currently considered as degraded thickets because of a lack of regeneration induced by increasing browsing pressure on desired tree species, under-canopy agriculture, overgrazing and collection of fuel wood, seeds, and ground fodder (Jazirehi & Ebrahimi 2003; Ghazanfari *et al.* 2004; Pourhashemi *et al.* 2004; Sagheb-Talebi *et al.* 2004).

Successful results in forest protection, conservation, and restoration, as well as in sustainable production of renewable natural resources are dependent on the current status of the ecosystems and using the suitable remedial measures (Abdollah Pour 1995). Wild almond as one of the valuable and rare species in Zagros ecosystem is a member of Rosaceae family and generally characterized by a green, vertical stems.

The plant has slender leaves and relatively big flowers. The egg-shaped fruit of the plant is covered with fluff and has a yellowish kernel. This plant is indigenous to south-west Asia and particularly to mountains and rocky areas of Iran.

The considerable reserves of oil and protein in their fruits promote several uses such as refreshments and manufacturing medicinal, cosmetic and hygiene products. In addition, this species can play an important role in the forest restoration and soil protection of arid and semi-arid regions (Rouhi *et al.* 2005; Alvani Nejad 1999).

The success of different management plans such as seed planting and sowing depends on the scientific information about plant responses (e.g. height, diameter and survival rate) to basic treatments such as seed pre-soaking and burial depth.

These findings from different conditions can be the basis of a guide for plantation of valuable species and restoration of degraded sites.

Physiographic factors affecting air temperature, humidity, evaporation and incident light should also be considered in plantation plans (Perring 1959) because they can affect photosynthesis and plant growth (DeLucia & Smith 1987). For example, a comparison between the northern and southern aspects of wild almond plantations showed that seedlings planted in the northern aspect had higher mean height, stem collar diameter and crown diameter (Iranmanesh & Jahanbazi Gojan 2007). Also, the study of 26 *Amygdalus* species suggests that altitude is the most important factor limiting the geographic distribution of this species (Browice & Zohary, 1995). The results of other studies indicated that seed physical treatments (Mirzadeh Vaghfi *et al.* 2009; Talebi *et al.* 2012) as was the case for soaking seeds in hot and cold water (Olmez *et al.* 2007) are able to impact their germination rate. Planting depth is another factor affecting seed germination and plant survival through its influence on soil moisture and temperature whose requirements differ among different species (Heydari *et al.* 2011).

Despite the importance of proper planting depth, this factor is rarely mentioned in seed planting guidelines.

In this study, we investigated the effects of some seed treatments on the survival and growth of *Amygdalus scoparia* after seed planting at different aspects and elevations under natural conditions. The results are able to provide basic information to suitable establishment of this important species in future projects.

## MATERIALS AND METHODS

### Site description

This study was carried out near Bagh-Malek city in the province of Khuzestan/South West of Iran, and included part of the Zagros region. The study area (50º 1′ 5″ to 50º 2' 50" N and 31º 30' 1″ to 31º 28' 48" E) is part of a multi-purpose forest management plan by Khuzestan natural resources office.

Mean annual precipitation is 590 mm and mean annual temperature is 17 °C with minimum and maximum of 9 and 32 °C, respectively. The elevation and slope of planting sites ranges from 1200 to 2000 m and 60 to 70 % respectively. The dominant tree species in the study area is oak (*Quercus persica*).

The treatment combination consisted of two factors, namely, physiographic and pretreatment methods.

The physiographic factor consisted of two elevations (1200 and 1800 m a.s.l.) and four aspects (north, south, east and west) for a total of eight planting sites.

In each planting sites, we established five completely randomized blocks within each of which a combination of two treatments was applied.

The first treatment was the seed burial depth (2, 4 or 6 cm), whereas the second treatment was seed pretreatment methods, namely, cold (normal) water soaking (10°C) and hot water (80°C) soaking for 24 hours.

Each of these six combinations of treatments was applied in six planting holes within each of which four seeds were planted for a total of 5760 planted seeds (2 elevations x 4 aspects x 5 blocks x 6 treatment combinations x 6 planting holes x 4 seeds).The planting holes were placed 1 m apart and their size was 20 x 20 x 20cm. The seed planting was conducted on December 15, 2010.

The required seeds were collected from naturally established elite trees located beside and within the experimental area (Table 1).

**Table1**. Characteristics of seeds.

| Purity (%) | Viability (%) | Seed source |
|---|---|---|
| 100 | 85 | Bagh-Malek |

Before planting, carboxin-thiram was used for seed disinfection (Golsam Gorgan, chemicals company, Iran). The impact of pretreatments was evaluated on the survival rate, height and diameter growth of newly emerged seedlings.

The characteristics of seedlings among the treatments were compared using One-way ANOVA and Duncan's multiple comparison tests (Diaz-Villa *et al.* 2003).

Residuals were examined with the Levene's test and Kolmogorov-Smirnov test to verify that the assumptions of homogeneity of variance and normality, respectively, were met. Also, the seedling characteristics between elevations and water temperature seed pre-treatments were compared using independent sample t-tests. All statistical analyses were performed using SPSS (version 16).

## RESULTS

### Seedling height

According to the ANOVA performed on seedling height, significant differences were detected between aspects, seed buried depth, aspect × altitude, and soaking × altitude (Table 2).

Based on Duncan's test, the height of seedlings established on the north-facing aspect was significantly higher than that of other aspects, while the lowest seedling height was observed in the south-facing aspect. Heights of seedlings established on east- and west-facing aspects had intermediate values and were statistically similar (Fig. 1).

The tallest seedlings were observed when the seeds were buried at a depth of 2 cm and seedling height tended to decrease with increasing burial depth.

The seedlings established on the north-facing aspect at both elevation levels (1200 and 1800 m) were significantly taller than those found on the south-facing aspect.

There were no significant differences in seedling height between seed pre-treatments (cold and hot water), but the highest seedling height was observed at 1800 m altitude by pre-soaking seeds in cold water (Table 2 and Fig. 1).

### Collar diameter

It can be seen from Table 3 that the differences in collar diameter among treatment types were statistically significant only for sowing depth treatments ($p<0.05$).

While the seedling collar diameter was significantly larger at 2 cm than at 4 and 6 cm burial depth, there was no significant difference between 4 and 6cm (Fig. 2).

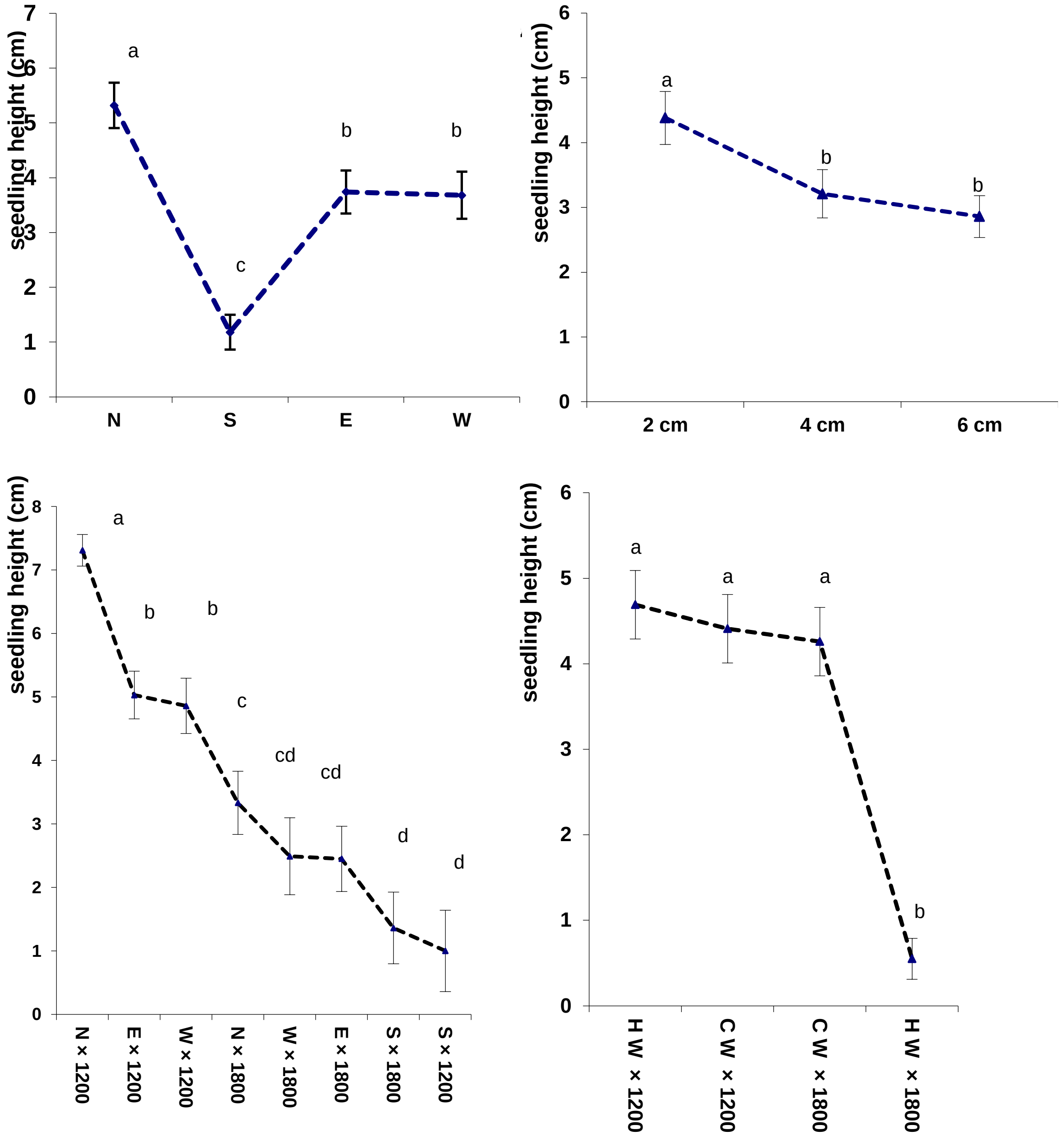


**Fig. 1.** The results of Duncan's multiple range test for seedling height (mean (± SE) in different treatments, different letters indicate significant differences. . . H W: Hot water, CW: Cold water.

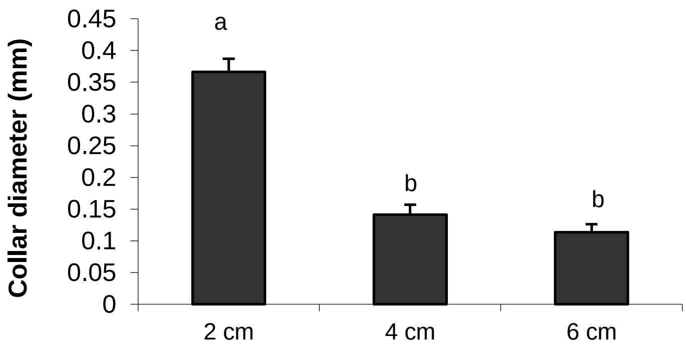


**Fig. 2**. The results of the Duncan's multiple range test for collar diameter (mean (± SE) of seedlings from different burial depths, different letters indicate significant differences.

Table 2. Results of the ANOVA performed between treatments in terms of seedling height.

| Source of variation | Mean Squares (ms) | Sums of squares (ss) | F | (df) | P |
|---|---|---|---|---|---|
| Aspect | 175.835 | 527.5 | 31.508 | 3 | **0.001 |
| Depth of sowing | 50.8 | 101.6 | 9.15 | 2 | **0.000 |
| Aspect × Soaking | 6.7 | 20.354 | 1.216 | 3 | ns 0.305 |
| Aspect × Depth of sowing | 0.833 | 4.995 | 0.149 | 6 | 0.898 ns |
| Aspect × Altitude | 49.66 | 149 | 8.9 | 3 | **0.000 |
| Soaking × Depth of sowing | 0.185 | 0.370 | 0.33 | 2 | ns 0.967 |
| Soaking × Altitude | 238 | 238 | 42.77 | 1 | **0.000 |
| Depth of sowing × Altitude | 3.055 | 6.11 | 0.547 | 2 | ns 0.579 |
| Aspect × Soaking × Depth of sowing | 6.319 | 37.21 | 1.32 | 6 | ns 0.345 |
| Aspect × Soaking × Altitude | 8.56 | 24.88 | 1.486 | 3 | ns 0.220 |
| Soaking × Depth of sowing × Altitude | 14.321 | 28.345 | 2.540 | 2 | ns 0.08 |
| Aspect × Depth of sowing × Altitude | 3.45 | 20.72 | 0.619 | 6 | ns 0.715 |
| Aspect × Soaking × Depth of sowing × Altitude | 1.659 | 10.16 | 0.304 | 6 | ns 0.934 |
| Error | 5.55 | 1071.21 | | 192 | |
| Total | | 5604 | | 240 | |

ns – Not significant; * – significant at α < 5%; ** – significant at α < 1%.

**Survival rate of seedlings**

Seedling survival was significantly affected by aspect, aspect × soaking, aspect × altitude, soaking × altitude, and aspect × soaking × altitude (Table 4).

Survival rate was higher on the north-facing aspect than other aspects and was lowest on the south-facing aspect.

Also, seed pre-soaking in cold water resulted in greater seedling survival compared to hot water. According to the significant aspect × soaking interaction, the survival rate of seedlings was higher in north-facing aspect after pre-soaking in cold water.

In fact, the survival rate of *A. scoparia* seedlings was higher in all facing aspects when the seeds were pre-soaked in cold water compared to hot water. At high altitude (1800 m), survival rate of seedlings whose seeds were pre-soaked in hot Water was significantly lower than that of seedlings whose seeds were pre-soaked in cold water. Interestingly, the survival rate of seedlings at low altitude (1200 m) whose seeds were pre-soaked in hot water was as high as that of the cold water seed pre-treatment at both altitudes (Fig. 3). The highest seedling survival rate was observed on the north-facing aspect at the altitude of 1800 m with seeds pre-soaked in cold water (Fig. 3). Survival rate was higher in 1200m than in 1800m altitude (Table 6).

Independent t-tests indicated that the seedling survival rate and height were significantly different between the two soaking treatments (Table 5). Both seedling characteristics were significantly higher in cold than in hot water (Fig. 4).

Also, both the survival rate and height of seedlings were significantly higher at an elevation of 1200 m than at 1800 m (Table 6 and Fig. 5).

**Table 3.** Results of the ANOVA performed between treatments in terms of collar diameter.

| **Source of variation** | **Mean Squares (ms)** | **sums** of **squares (ss)** | **F** | **(df)** | **P** |
|---|---|---|---|---|---|
| Aspect | 0.66 | 1.98 | 1.62 | 3 | 0.186 ns |
| Depth of sowing | 1.56 | 3.07 | 3.77 | 2 | 0.02* |
| Soaking × Aspect | 0.356 | 1.09 | 0.895 | 3 | 0.455 ns |
| Depth of sowing × Aspect | 0.274 | 1.67 | 0.672 | 6 | 0.61 ns |
| Altitude × Aspect | 0.477 | 1.34 | 1.09 | 3 | 0.351 ns |
| Depth of sowing × Soaking | 0.542 | 1.08 | 1.33 | 2 | 0.26 ns |
| Altitude × Soaking | 0.176 | 0.176 | 0.432 | 1 | 0.512 ns |
| Altitude × Depth of sowing | 0.110 | 0.22 | 0.271 | 2 | 0.761 ns |
| Depth of sowing × Soaking × Aspect | 0.361 | 2.16 | 0.886 | 6 | 0.5 ns |
| Altitude × Soaking × Aspect | 0.473 | 1.42 | 1.16 | 3 | 0.325 ns |
| Altitude × Depth of sowing × Soaking | 0.02 | 0.05 | 0.06 | 2 | 0.93 ns |
| Altitude × Depth of sowing × Aspect | 0.4 | 2.43 | 1.004 | 6 | 0.424 ns |
| × Depth of sowing × Soaking × Aspect Altitude | 0.469 | 2.8 | 1.15 | 6 | 0.334 ns |
| Error | 0.407 | 78.19 | | 192 | |
| Total | | 108/110 | | 240 | |

ns – Not significant; * – significant at α < 5%; ** – significant at α < 1%

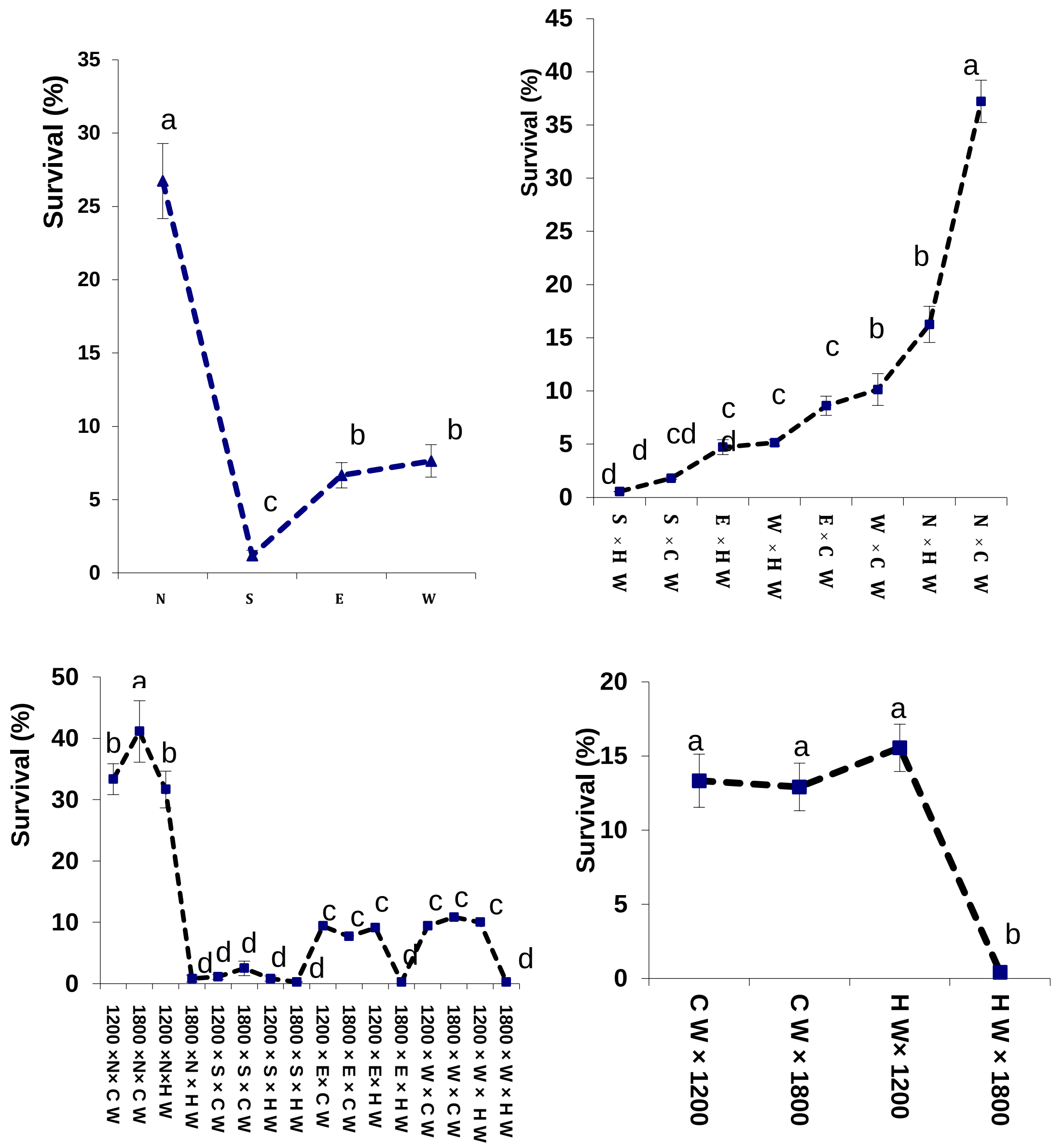


**Fig. 3**. Results of Duncan's multiple range tests for survival rate of seedlings (mean (± S.E.) in different treatments, different letters indicate significant differences. H W: Hot water, CW: Cold water.

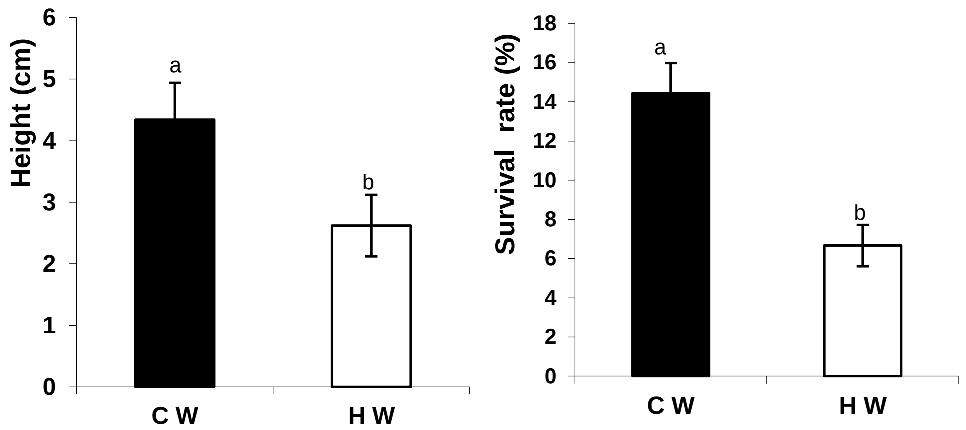


**Fig. 4.** Mean values of survival rate and seedling height in two presoaking in water. HW: Hot water, CW: Cold water.

**Table 4.** Results of the ANOVA between treatments in terms of survival rate of seedlings.

| Source of variation | Mean Squares (ms) | sums of squares (ss) | F | (df) | P |
|---|---|---|---|---|---|
| Aspect | 4766 | 223399 | 108.25 | 3 | **0.000 |
| Depth of sowing | 192.34 | 384.69 | 2.78 | 2 | ns 0.062 |
| Aspect × Soaking | 1179.72 | 3593 | 17.36 | 3 | **0.000 |
| Aspect × Depth of sowing | 28.47 | 170.86 | 0.413 | 6 | ns 0.87 |
| Aspect × Altitude | 363 | 1089 | 5.26 | 3 | **0.002 |
| Soaking × Depth of sowing | 22.46 | 45.21 | 0.328 | 2 | ns 0.721 |
| Soaking × Altitude | 3251.15 | 3251 | 47.21 | 1 | **0.000 |
| Depth of sowing × Altitude | 21.77 | 43.26 | 0.326 | 2 | ns 0.730 |
| Aspect × Soaking × Depth of sowing | 30.26 | 181.26 | 0.439 | 6 | ns 0.852 |
| Aspect × Soaking × Altitude | 1004 | 3012.26 | 14.55 | 3 | **0.000 |
| Soaking × Depth of sowing × Altitude | 93.23 | 186.77 | 1.35 | 2 | ns 0.261 |
| Aspect × Depth of sowing × Altitude | 16 | 96.49 | 0.323 | 6 | ns 0.965 |
| Aspect × Soaking × Depth of sowing × Altitude | 5.71 | 28.34 | 0.08 | 6 | ns 0.99 |
| Error | 68.97 | 13243 | | 192 | |
| Total | | 79687 | | 240 | |

ns – Not significant; * – significant at α < 5%; ** – significant at α < 1%

**Table 5.** Results of independent t- tests between soaking treatments in terms of height, collar.

| Source of variation | Cold water | Hot water | d.f | F | t | P |
|---|---|---|---|---|---|---|
| Seedling height | 4.34 | 2.62 | 238 | 1.97 | 4 | **0.000 |
| collar diameter | 0.21 | 0.2 | 238 | 2.87 | 0.15 | ns 0.8 |
| survival rate | 14.44 | 6.66 | 238 | 18.13 | 4.18 | **0.000 |

Diameter, height and survival rate (mean (± S.E.) of seedlings

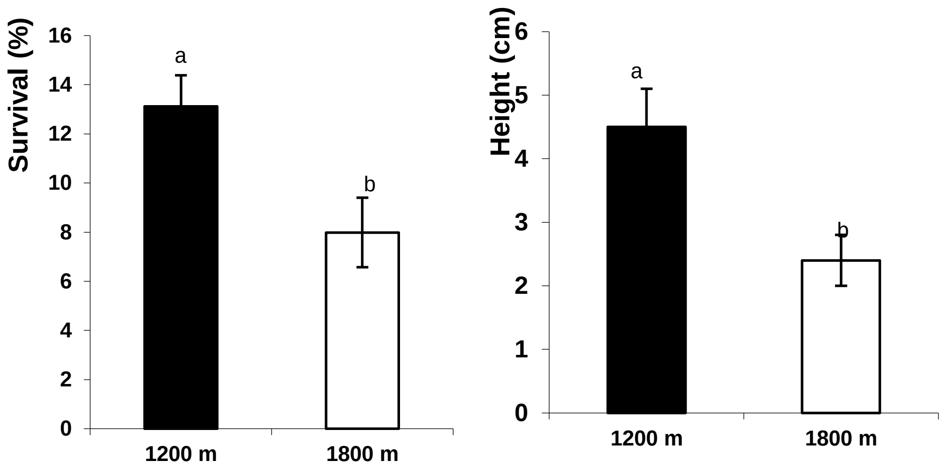


**Fig. 5.** Mean values of survival rate and seedling height in two altitude ranges treatment.

**Table 6.** Results of independent t-tests between altitude ranges in terms of height, collar.

| Source of variation | 1800 m | 1200 m | df | F | t | P |
|---|---|---|---|---|---|---|
| Seedling height | 2.4 | 4.5 | 238 | 3.1 | 5 | **0.000 |
| collar diameter | 0.18 | 0.22 | 238 | 1.36 | 0.47 | ns 0.6 |
| survival rate | 7.98 | 13.12 | 238 | 0.09 | 2.7 | **0.007 |

Diameter, height and survival rate (mean (± SE) of seedling

## DISCUSSION

For several species including *A. scoparia*, information about seed dormancy is limited (Khalil & Al-Eisawi 2000; Salarian *et al.* 2008; Rouhi *et al.* 2009; Rahemi *et al.* 2009). Results showed that seedling height, survival rate and, to a lesser extent, collar diameter had highest values on north-facing aspects and lowest values on south-facing aspects. Thus, aspect is likely an important factor in the establishment of *A. Scoparia* seedlings, because of related microclimatic effects. Aspect has a major influence on the amount of incident solar radiation which can affect plant photosynthesis and phenology (Barnes *et al.* 1998; Austin, 2005) and productivity (Desta *et al.* 2004). In the northern hemisphere, the duration and intensity of solar radiation are higher on south-facing aspects, resulting in a relatively warmer and drier microclimate and more accentuated seasonal environmental extremes (Holst *et al.* 2005). On the other hand, slope aspects can affect soil nutrient status (Kelemmedson & Wienhold 1992; Sharma *et al.* 2010). Microclimatic affects north-facing aspect providing advantages to the establishment of *A. scoparia,* include improved moisture availability and lower temperatures. The larger exposition to solar radiation of south aspects can be a serious issue in arid and semi-arid regions, such as our study area. Indeed, moisture is one of the most important factors in regeneration establishment in arid and semi-arid regions and the success of regeneration depends largely on the ability of plant roots to access soil moisture (Shafroth *et al.* 2000). South-facing aspect has warmer and drier microclimate, so that soil moisture is generally lower than on other aspects (Rosenberg *et al.*, 1983). Soils of northern slopes in the Zagros region were found to be deeper and more fertile than those of other aspects (Heidari *et al.* 2011). Accordingly, in the Mishan region, west of Iran, the mean height, collar diameter, crown diameter and number of coppice sprouts on north aspect were significantly larger than those measured on the south aspect (Iranmanesh & Jahanbazi Gojani 2007). AlvaniNejad (1999) also concluded that aspect is one of the most important factors in the distribution and the establishment of *A. scoparia* in the Zagros region.

The tallest and largest diameter seedlings were observed from the seeds buried at a depth of 2cm and these characteristics significantly decreased with increasing burial depth. Although a deeper burial usually prevents seed predation, it also delays the seedling emergence (Tomlinson *et al.* 1997). Seed germination and seedling emergence and development are thus reduced with increasing seed burial depth. The main reason for the low germination of seeds deeply buried is the induction of secondary dormancy. Seed dormancy is caused by a slowdown of the gas exchange which is more accentuated with increasing seed burial depth (Asgharipour 2011). Aside from seed secondary dormancy, the lower seedling emergence of deeper buried seeds can be associated with seed destruction and early germinated seedlings (Benvenuti & Macchia 1998). Environmental factors such as soil water content, light and temperature may limit the germination of buried seeds (Pereja & Staniforth 1985). The interaction of factors such as low light, reduced gas exchange and presence of $CO_2$ produced by soil biological activities (overall unsuitable conditions) can reduce seed growth and vitality (Asgharipour 2011). Our results in this regard are similar to those of Heidari *et al.* (2011) in Northern Zagros on *Quercus brantii*, Sewia *et al.* (2002) on Japanese Chestnut (*Castanea crenata*) and Gholomi *et al.* (2007) on *Pistacia atlantica*. These results suggest that there is an optimal range of burial depth to maximize seedling emergence and subsequent seedling development.

Using physical and chemical treatments such as seed soaking in water, moist chilling, and scarification by sand paper, sulfuric acid, potassium nitrate are useful to overcome seed dormancy of many species (Zoghi *et al.* 2011). The embryo of many seeds fails to germinate because of a lack of oxygen diffusion through the seed coat. At cold temperatures, more oxygen is soluble in water and the oxygen requirements of the embryo are more easily satisfied. Pre-soaking at high temperature can produce negative effects by causing a decline in seed germination and damage to the seed embryo (Nabaee *et al.* 2013). On the other hand, seeds exposed to low temperature achieve chilling requirement and probably increased endogenous GA3 level that can have positive effect on germination and growth (Rouhi *et al.* 2005). These authors showed that germination rate of seed and seedling length of *A. scoparia* were higher at 7 °C than at 22 °C.

We found significant differences between two altitude levels in terms of seedling survival rate and height. These characteristics were significantly higher at the altitude of 1200 m than 1800 m. These results are supported by those of a study about effective factors on wild almond distribution in the Markazi Province of Iran within which the maximum canopy cover and regeneration density was observed at the altitude of 1000-1500 m as compared to 1500-2000 m (Goodarzi *et al.* 2012).

## CONCLUSION

North-facing aspects were more suitable for seeding of *A. scoparia*. The use of cold water as a seed pre-treatment in conjunction with a north-facing seeding at a burial depth of 2 cm produced the best results in terms of seedling survival. Therefore, we do not recommend performing a south-facing seeding of wild almond. Instead, a north-facing seeding at higher altitude with pre-soaked seeds in cold water is more likely to produce successful establishment of *A. scoparia* in the field. Alternatively, a seeding under the same conditions, but at an altitude of 1200 m, can also produce suitable results.

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# اثر پیش تیمار خیساندن بذر، عمق کاشت و شرایط رویشگاه بر زنده‌مانی و رشد بادام وحشی (*Amygdalus scoparia*)

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**چکیده**

بادام وحشی (*Amygdalus scoparia*) از مهم‌ترین گونه‌ها است که کارکردهای متنوع اکولوژیکی را در اکوسیستم جنگلی زاگرس دارد. در این مطالعه، ما اثرات برخی پیش‌تیمارهای بذری را بر روی زنده‌مانی و رشد بادام کوهی بعد از کاشتن بذر در جهت‌های مختلف دامنه و ارتفاع از سطح دریا تحت شرایط طبیعی بررسی کردیم. پیش تیمارهای بذر شامل خیساندن بذرها در آب سرد (۱۰ درجه سانتی‌گراد) و آب گرم (۸۰ درجه سانتی‌گراد) به مدت ۲۴ ساعت بود. اثر این پیش تیمارها بر درصد زنده‌مانی، رشد ارتفاعی و قطری نهال‌های تازه جوانه زده ارزیابی شد. بر اساس آزمون دانکن، ارتفاع نهال‌های مستقر شده در جهت دامنه شمالی به طور معنی‌داری نسبت به سایر دامنه‌ها بیشتر بود، درحالیکه کمترین ارتفاع نهال‌ها در جهت جنوبی مشاهده شد. ارتفاع نهال‌های مستقر شده در جهات دامنه شرقی و غربی در حد واسط بود و از نظر آماری مشابه بودند. بلندترین نهال‌ها در حالتی که بذرها در عمق ۲ سانتی‌متر کاشت شده بودند مشاهده شد و ارتفاع نهال‌ها با افزایش عمق تمایل به کاهش داشت. نهال‌های مستقر شده در جهت دامنه شمالی در هر دو دامنه ارتفاعی (۱۲۰۰ و ۱۸۰۰ متر) به طور معنی‌داری نسبت به نهال‌های مستقر شده بر دامنه جنوبی بلندتر بودند. به طور کلی، استفاده از آب سرد به عنوان یک پیش تیمار در ترکیب با کاشت نهال در دامنه شمالی و عمق ۲ سانتی‌متر زنده‌مانی و توسعه نهال‌های بادام کوهی را به حداکثر میزان رساند.

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