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The impacts of altitude and seed pretreatments on seedling emergence of Syrian juniper (*Juniperus drupacea* (Labill.) Ant. et Kotschy)

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Abstract

Background: Syrian juniper is an economically important species and in danger of extinction. For these reasons, the best seedling production methods of the species should be determined and its plantations should be established. The aim of the study is to examine the impacts of altitude and different pretreatment combinations of cold and warm stratifications, citric acid, shaking seeds in bottles with crushed glass, and different stimulating agents including Baikal EM1, Biohumus, Polystimulin A6 and K hormones (PS-A6 and PS-K) on seed germination of Syrian juniper. To conduct the study, the cones of the Syrian junipers were collected from three different altitudes (1000, 1200, and 1400 m a.s.l.) within the boundaries of Forest Management Directorate of Bozyazı, Mersin in Turkey. The seeds were subjected to three replicates per one treatment (altitude in interaction with pretreatment). There were 100 seeds per replicate.

Results: Two-way ANOVA revealed significant effects of altitude, pretreatment, and their interaction on seedling emergence of Syrian juniper. Seeds from higher altitudes had higher germination rates in all pretreatments. The highest percent emergence (85%) was recorded in the combination consisting of shaking with crushed glass, both cold and warm stratification, and successive application of stimulators PS-A6 and PS-K. All the treatments with most germinating seeds encompassed shaking in crushed glass or soaking in citric acid, both warm and cold stratifications, and application of stimulating agents.

Conclusions: The use of seeds from higher altitudes should be considered for seedling production of Syrian juniper due to their higher germination potential. Beyond altitudinal differences, specific pretreatments can rise germination potential more than twofold.

Keywords: Auxin, Citric acid, Cytokine, Germination, Stratification

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Background

Today, *Juniperus drupacea* forests are in danger of extinction and the contraction process of their forests continues. In Turkey, vegetative and generative sapling production technique, afforestation, and natural rejuvenation techniques of stubble are still not fully determined. Understanding eco-physiological limits to seed germination is a pre-requisite for successful plantation on a large scale. Without it, neither massive seedling production could be realized nor new plantations could be created. *J. drupacea* forests are under heavy grazing pressure. Their forests are being destroyed to obtain tar, which is sold at very high prices. *Juniperus* molasses, which was consumed only by the local people, was transformed into a commercial material with the increase of tourism, and the Juniper forests have become adversely affected as a result. In order to prevent the extinction of the economically important species, both the best seedling production methods should be determined and the plantations of the species should be established. Seedling regeneration is complicated by seed deep dormancy of the species.

Reproductive biology, particularly germination characteristics, is crucial to understanding how species cope with environmental variation (Baskin and Baskin 2014; Bauk et al. 2017) because it could vary depending on altitude and other factors. Juniper seedling regeneration occurs even at high altitudes where the establishment and growth of plants occur in short periods. Some species can recruit through rapid germination during less severe climatic periods (Archibold 1989). A species may extend over the large geographical area and can grow in a wide range of environments with varying climates and topography. It usually has populations adapted to the different local environmental conditions (Ghildiyal et al. 2009). Therefore, a comparison of the germination capacity of seeds from different altitudes is relevant to determine the reproductive efficiency of a species (Vera 1997).

Seed dormancy in plants can be split into five basic classes including physiological, morphological, morphophysiological, physical, and combinational (physical and physiological) dormancies (Baskin and Baskin 2004). Syrian juniper' seeds, which have an underdeveloped embryo with a physiological component of dormancy (Baskin and Baskin 2004), have morphophysiological dormancy (Yavuz and Yılmaz 2017). Up to date, several scientific studies (Gültekin et al. 2004; Gültekin et al. 2005; Gürlevik and Gültekin 2008; Yavuz and Yılmaz 2017) performing warm and cold stratification, treatment with sulfuric acid, gibberellic acid, mechanical treatment, cracking, and different sowing times have been conducted to enhance the seed germination of Syrian juniper. Kirdar and Ertekin (2008) reported that cytokinin-like polystimulin-K and bioactive polystimulin-A6 mostly have positive effects on breaking dormancy and enhancing germination. However, in the earlier studies mentioned above, pretreatments have never been combined together for the seeds from different altitudes. Therefore, in this study, the seeds of Syrian juniper from three different altitudes were subjected to warm and cold folding and mechanical treatment, which have been tried in previous studies to combat germination of stubble seeds, different concentrations of citric acid and hormones.

Materials and methods

Species description

Syrian juniper (*Juniperus drupacea* Ant. et Kotschy, Cupressaceae) is known as "Andız" in Turkey. This species is similar to junipers in terms of many characteristics, but its seeds are not free and buds are covered with scales (Yaltırık 1993). It grows up to about 25 m in height with 100 cm diameter of breast height and has typically a pyramidal crown (Gültekin 2007). The species, native to the Taurus and Anti-Taurus Mountains of the Mediterranean Region in Turkey, is mainly diffused in South Anatolia, Syria, Lebanon, and Anti-Lebanon (Yaltırık 1993). Its altitudinal distribution is between 600 and 1800 m in Turkey (Gültekin 2007) with cold winters and dry summers and is indifferent to soil type as it can grow on calcareous or granitic soil (Farjon 2010).

Syrian juniper generally occurs with *Juniperus excelsa* Bieb., *J. foetidissima* Willd, *Cedrus libani* A. Rich., *Pinus nigra* Arnold., *Pinus brutia* Ten., *Abies cilicica* Carr., *Quercus* L., and various shrub species (Gültekin 2007). This is a heliophilous tree with better growth in relatively open forests, provided a certain level of humidity in order to thrive. The Syrian juniper can be used to enhance the biodiversity in oak and cedar forest restoration (Douaihy et al. 2017). Furthermore, it plays a crucial role in the medicinal (Akbulut et al. 2008; Miceli et al. 2011; Akbulut 2015), cosmetic, social and environmental fields (Sa et al. 2017), in human nutrition (Akbulut et al. 2008), for foods and beverages as a natural antioxidant supplement (El-Ghorab et al. 2008), and as an ornamental tree (Farjon 2010; Yavuz and Yılmaz 2017). On the other hand, *Juniperus* is one of the trees that are resistant to extreme climatic and soil conditions and have a deep taproot. Due to these features, they are primarily versatile wood species used in the unproductive forest areas and the wind and snow curtains (Gültekin et al. 2004).

The cone mass of Syrian juniper leads to 134 cones per kilogram. The average number of healthy embryos in seeds was 2 (Gültekin et al. 2005).

Materials

The cones were collected from natural stands at three different altitudes (1000, 1200, and 1400 m a.s.l.) within the boundaries of Forest Management Directorate of Bozyazı, Mersin, in November 2017. The cones were randomly taken from 30 trees of each altitude that were at least 50 m apart. About 200 cones were collected for each tree spreading all over the crown and then were mixed depending on the altitude. The cones were pretreated; they were kept moist in a 4 °C refrigerator without being desiccated for 20 days. The seeds were extracted from cones prior to being subjected to pretreatments.

Methods

The seeds were subjected to three replicates per one treatment (altitude in interaction with pretreatment). There were 100 seeds per replicate. The pretreatment combination consisted of two factors, namely, altitude and pretreatment methods. The experiment included 18 treatments combining two factors. The first pretreatment (I) was a control without being applied any further manipulation (Table 1). Prior to the experiment, the viability of non-germinating seeds was checked using the float test.

The morphological dormancy was removed either by shaking of seeds in bottle with crushed glass for 2 h (BCG) or by soaking in 1000 mg L^{-1} citric acid ($C_6H_8O_7$) for 100 min at 22–24 °C (SCA). To break the morpho-physiological dormancy, either cold stratification for 220 days at 3 °C (CS) or warm stratification for 65 days at 35 °C (WS) was done. To stimulate the seeds to germinate, soaking in 700 mg L^{-1} Baikal EM1 for 25 days (SBE), soaking in 700 mg L^{-1} Biohumus for 25 days (SBH), soaking in 700 mg L^{-1} polystimulin A6 for 25 days (SPA), and soaking in 700 mg L^{-1} polystimulin K for 25 days (SPK) were used (Table 1).

Seeds were stratified in a beaker containing sand moistened with distilled water and stored in a refrigerator at 3 °C (cold stratification). They were incubated at 35 °C for warm stratification. Polystimulins (PSs) are the synthetic high-molecular-weight plant growth regulators. PS-A6 and PS-K with high biological activity are similar to auxin and cytokinin, respectively. They are described as plant growth regulators (Tsatsakis et al. 1993).

Germination tests were carried out in a greenhouse where the mean temperature and humidity were 26 ± 0.5 °C and 80 ± 5% respectively in October 2018. Mean soil temperature was 21 ± 0.5 °C. Photoperiod was 11 h each day. Seeds were sown in sandy-clay loam at a depth

Table 1 Overview of pretreatment methods. All of them were applied separately to seeds originating from 1000, 1200, and 1400 m a.s.l., respectively

Treatment no.	Removal of morphological dormancy		Stratification		Stimulator			
	BCG	SCA	CS	WS	SBE	SBH	SPA	SPK
I (control)								
II	x		x					
III	x			x				
IV	x		x		x			
V	x			x		x		
VI	x		x	x	x			
VII	x		x	x	x	x		
VIII	x		x				x	
IX	x			x				x
X	x		x	x			x	x
XI		x	x					
XII		x		x				
XIII		x	x		x			
XIV		x		x		x		
XV		x	x	x	x	x		
XVI		x	x				x	
XVII		x		x				x
XVIII		x	x	x			x	x

BCG—shaking of seeds in bottle with crushed glass for 2 h, CS—cold stratification for 220 days at 3 °C, WS—warm stratification for 65 days at 35 °C, SCA—soaking in 1000 mg L^{-1} citric acid ($C_6H_8O_7$) for 100 min at 22–24 °C, SBE—soaking in 700 mg L^{-1} Baikal EM1 for 25 days, SBH—soaking in 700 mg L^{-1} Biohumus for 25 days, SPA—soaking in 700 mg L^{-1} polystimulin A6 for 25 days, SPK—soaking in 700 mg L^{-1} polystimulin K for 25 days as twice as its size. The percent emergence of seedlings for each pretreatment was calculated by dividing the total number of seeds that germinated in each pretreatment by the number of seeds sown (excluding dead seeds) and multiplied by 100. Data were transformed using Arcsin square root and then analyzed using the model of two-way ANOVA. Statistical analyses were performed by using SPSS version 22.

Results

Two-way ANOVA revealed significant differences for altitude (F = 425.127; df = 2), pretreatment (F = 121.412; df = 17), and significant interaction of altitude and pretreatment (F = 6.428; df = 34; df_{error} = 108) at the 0.001 probability level. All sources of variation had an effect on the percent emergence with altitude as the main factor.

The mean percent emergence increased by as much as 20% from the lowest (1000 m) to the highest altitude, and altitude rankings were practically general among pretreatments, with the only exception of pretreatment VII consisting of shaking with crushed glass, both cold and warm stratifications, and successive applications of stimulators Baikal EM1 and Biohumus, respectively (Table 2, Fig. 1).

Table 2 Mean values and standard errors for percent emergence (%) of the seedlings for pretreatments (PTs)

PTs	Altitude (m)			Overall
	1000	1200	1400	
	Mean[a] ± standard errors			
I (control)	20.5 ± 0.3kl	30.3 ± 2.7g	44.1 ± 3.7fg	31.6 ± 3.7
II	22.4 ± 1.4jkl	35.9 ± 2.4fg	45.1 ± 1.9efg	34.5 ± 3.4
III	26.2 ± 1.2ghj	33.9 ± 1.9fg	47.5 ± 2.5def	35.9 ± 3.3
IV	24.1 ± 1.6hjk	37.6 ± 3.7efg	43.5 ± 3.5fg	35.1 ± 3.3
V	33.8 ± 1.8ef	35.6 ± 2.2fg	45.4 ± 2.9efg	38.3 ± 2.2
VI	41.7 ± 1.9c	48.5 ± 2.0c	78.5 ± 1.4ab	56.2 ± 5.7
VII	52.0 ± 1.0b	67.9 ± 1.4b	49.1 ± 1.9cdef	56.3 ± 3.0
VIII	30.3 ± 1.0fg	41.5 ± 2.7cdef	46.1 ± 2.6efg	39.3 ± 2.6
IX	23.8 ± 1.9hjk	44.7 ± 2.4cde	43.9 ± 2.1fg	37.5 ± 3.6
X	58.1 ± 1.5a	75.5 ± 1.8a	84.6 ± 0.6a	72.7 ± 4.0
XI	18.2 ± 1.7 l	35.4 ± 2.2fg	38.5 ± 1.5gh	30.7 ± 3.3
XII	11.6 ± 0.6m	30.1 ± 2.4g	35.5 ± 2.2h	25.7 ± 3.7
XIII	38.5 ± 1.0cd	46.0 ± 3.3cd	54.1 ± 3.1cd	46.1 ± 2.6
XIV	34.8 ± 2.6de	48.5 ± 1.5c	56.4 ± 2.9c	46.6 ± 3.4
XV	51.5 ± 0.7b	68.0 ± 1.6b	74.6 ± 1.6b	64.7 ± 3.5
XVI	30.3 ± 0.6fg	40.8 ± 2.5def	46.5 ± 3.3def	39.2 ± 2.7
XVII	27.9 ± 1.4gh	38.7 ± 1.5def	52.5 ± 1.7cde	39.7 ± 3.6
XVIII	52.4 ± 0.6b	66.0 ± 2.5b	77.8 ± 1.7ab	65.4 ± 3.8
Overall	33.2 ± 1.8	45.8 ± 1.9	53.5 ± 2.0	

[a]Means within each column followed by the same letter are not significantly different ($p < 0.05$)

Across altitudes, the pretreatments were able to change germination rates more than twofold (Table 2 and Fig. 1). The highest percent emergence within all altitudes was recorded for pretreatment X consisting of shaking with crushed glass, both cold and warm stratifications, and successive applications of stimulators PS-A6 and PS-K, respectively. The combinations of cold or warm stratifications with shaking in crushed glass or soaking citric acid exhibited low emergence.

Discussion

We found a high effect of altitude in the performance of seeds of Syrian juniper. Besides, the pretreatments analyzed were able to enhance germination considerably in this species.

The percent emergence of Syrian juniper seedlings differed considerably among altitudes, such that seeds from high-altitude populations germinated more than those from low-altitude populations. Hence, Syrian juniper exhibited higher germination in seeds from 1400 m. Likewise, Douaihy et al. (2017) reported that optimal altitude for *J. drupacea* was between 1500 and 1600 m. Different responses in the seed germination percentage of other species along an altitudinal gradient were described. Seed germination percentage increased with the rising altitude in *Calluna vulgaris* and *Erica cinerea* (Vera 1997), *Terminalia chebula* and *T. tomentosa* (Chauhan et al. 2007), *Pinus roxburghii* (Ghildiyal et al. 2009), and *Quercus leucotrichophora* (Saklani et al. 2012).

In previous studies, the highest germination percentages for the same species were reported as 83% with sowing of excised embryos following stratification over 45 days at 11–15 °C (Gültekin et al. 2005), 81% with the combination of warm stratification for 4 weeks and cold stratification for 9 weeks (Yavuz and Yılmaz 2017), 66% with cracking (Gürlevik and Gültekin 2008), and 53% with the combination of cracking and summer sowing (Gültekin et al. 2004).

Although cold and warm stratifications applied for embryo growth/radicle emergence require a considerably long period of time (Baskin and Baskin 2004), in fact, the current study and earlier studies have revealed that only prolonged stratification was not enough in seeds with morphophysiological dormancy. For instance, Yavuz and Yılmaz (2017) found that only 33% germination percentage in seeds of species was obtained from cold stratification for 16 weeks at 4 °C. In turn, either seeds of species should be sown after cold stratification over 8 weeks at 10–15 °C as suggested by Gültekin et al. (2005) or seeds of species should be sown after being applied a combination of cold and warm stratifications and other pretreatments by considering the results from the current study.

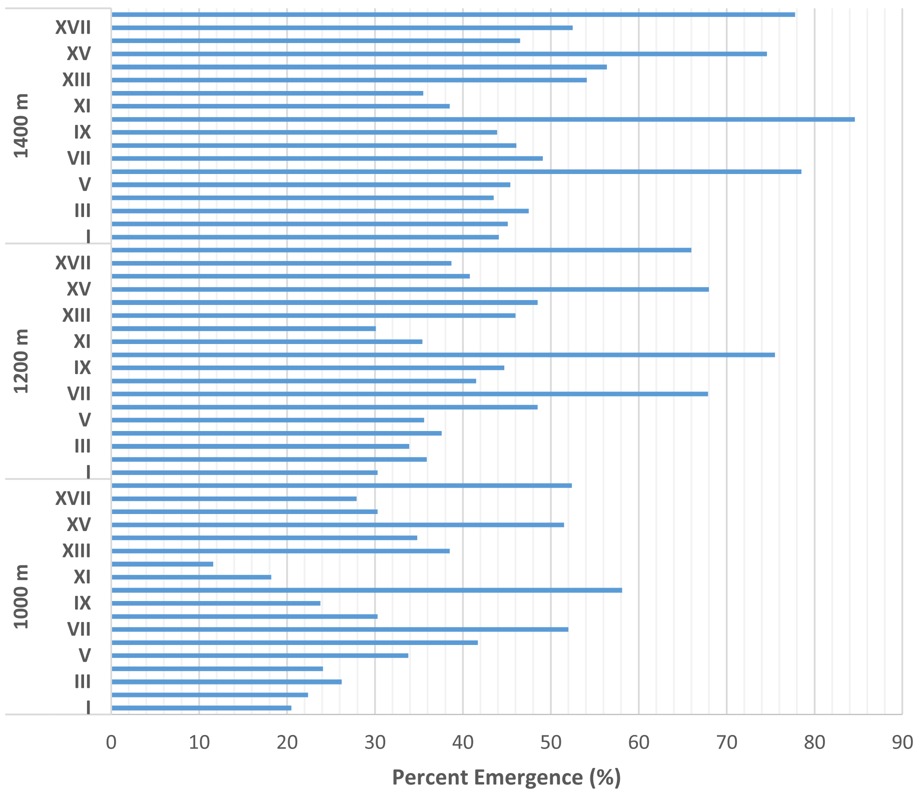

Fig. 1 Percent emergence of the seedlings by altitudes and pretreatments (control is the number I)

Pretreatment-XVIII consisting of soaking in citric acid, both cold and warm stratifications and successive applications of stimulators PS-A6 and PS-K, respectively showed one (78%) of the highest percent emergence in the current study. In a similar way, Yavuz and Yılmaz (2017) reported that the seeds pre-chilled for 8 weeks and soaked with 500-ppm gibberellic acid also demonstrated high germination percentage (64%). Unlike these results, the same species' seeds soaked both in sulfuric acid (Gültekin et al. 2005) and gibberellic acid (Yavuz and Yılmaz 2017) without another pretreatment had no germination. Tilki (2004) also stated that gibberellic acid had little effect on the germination of seeds of *Laurus nobilis* without pericarp but significantly increased the overall germination when used together with cold stratification.

All the treatments with most germinating seeds encompassed both warm and cold stratification. Therefore, it would be appropriate to apply different pretreatment combinations to remove the germination barrier of the species seeds. However, it should not be forgotten that although these combinations involving seed cracking processes yielded high yield percentages, it will be more practical and efficient to apply citric acid soaking as seed cracking requires intense labor and there is a possibility of damaging seed viability during cracking.

Conclusions

The results of this research have implications for the propagation of Syrian juniper. To obtain uniform germination and higher percent emergence of the seedlings in the nursery, high altitude populations (1400 m) combined with bottle-shaking with crushed glass, long cold and warm stratifications (220 and 65 days, respectively), plus applying bio-stimulators (PS-A6 + PS-K for 25 days) should be used. Moreover, seeds from higher altitudes should be considered as a factor to achieve higher germination potential.

Finally, further research is needed, since seed-source altitude may have an effect not only on germination but also on growth and seedling mortality depending on the altitude of the plantation area due to genetic adaptation. Beyond altitudinal differences, pretreatment can rise germination more than twofold.

Abbreviations

PS-A6: Polystimulin A6 hormones similar to auxin; PS-K: Polystimulin K hormones similar to cytokinin; Baikal EM1: Hormones; Biohumus: Hormones; BCG: Bottle with crushed glass; CS: Cold stratification; WS: Warm stratification; SCA: Soaking in 1000 mg L^{-1} citric acid ($C_6H_8O_7$); SBE: Soaking in 700 mg L^{-1} Baikal EM1 for 25 days; SBH: Soaking in 700 mg L^{-1} Biohumus for 25 days; SPA: Soaking in 700 mg L^{-1} PS-A6 for 25 days; SPK: Soaking in 700 mg L^{-1} PS-K for 25 days

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Authors' contributions
CY, MC, HBO, AESAA, OBMA, AMOA analyzed and interpreted the data. CY performed the examination of the study, and was a major contributor in writing the manuscript. CY, MC, HBO, AESAA, OBMA, AMOA analyzed the data, and wrote the manuscript. materials, AESAA, OBMA, AMOA made data collection and/or processing. All authors read and approved the final manuscript.

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