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Germination responses of oriental spruce (*Picea orientalis* (L.) Link) seeds from different provenances to thermal treatments after long-term storage

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Abstract: Oriental spruce (*Picea orientalis* (L.) Link) is a relict–paleoendemic species that requires priority conservation due to its ecological and genetic significance. In this study, changes in germination percentage (GP) and mean germination time (MGT) were evaluated following 11 years of storage at 0–4 °C in seeds collected from 20 populations representing the natural distribution range of the species. The effects of thermal pretreatments, including hot water (40 °C) and dry heat (90 °C), on these parameters were also assessed. Statistical analyses revealed that seed origin was the primary factor shaping germination performance, with GP values varying widely among populations, ranging from 10.04% to 78.04%. The highest germination percentage (78.04%) was recorded in the Dereli 1350–1650 m a.s.l. population. MGT values ranged from 9.88 to 15.56 days, with the longest germination time observed in the Karadağ 1650–1950 m a.s.l. population. Among the thermal experimental variants, the 90 °C dry heat treatment significantly reduced germination success, whereas the 40 °C hot water treatment produced more balanced results, closer to the control group. In addition, increasing elevation had a significant negative effect on germination success, and germination time was prolonged in high-elevation populations. Despite its limited distribution and relict–paleoendemic nature, the ability of this species to maintain a certain level of seed viability even after 11 years of storage represents a significant advantage for ex situ conservation strategies.

Keywords: storage duration, provenance, genetic erosion, thermal pretreatments, variation

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Introduction

Oriental spruce (*Picea orientalis* (L.) Link) is a relict–paleoendemic species with a limited distribution in the Eastern Black Sea Region of Türkiye and requires priority conservation due to its ecological and genetic significance. The natural distribution range of the species exhibits high topographic and climatic heterogeneity, and considerable variation in cone and seed characteristics has been reported among populations (Atalay, 1984; Boydak, 1996). The physiological and morphological traits of Oriental spruce seeds vary depending on microclimatic conditions of their origin, and significant differences in germination capacity and viability potential have been observed among populations (Yahyaoğlu & Genç, 2007; Özpay & Boydak, 1995).

Oriental spruce is considered one of the priority tree species for conservation due to its limited natural distribution worldwide and its genetic and ecological importance (Güney et al., 2019). Ecologically, it is a dominant species in the subalpine zone of the Northeastern Black Sea Region of Türkiye and the Caucasus, where it forms resistant forest stands near the timberline and plays a critical role in maintaining ecosystem balance through its contributions to biodiversity, soil protection, and watershed stability (Üçler et al., 2018). Oriental spruce also has considerable economic importance due to the wide range of uses of its wood in construction timber, furniture production, paper and cellulose industries, and musical instrument manufacturing, as well as its frequent use as an ornamental species in landscape architecture because of its aesthetic form. In addition, the demand for Oriental spruce timber raw material has been continuously increasing. The species also contributes significantly to forest-based livelihoods and social welfare in the region. Owing to its restricted distribution, climate-sensitive distribution characteristics, ecological role, and economic value, the conservation and sustainable management of its genetic resources are of particular importance (Acarer, 2024; Turna, 2004).

Proper seed storage is a critical prerequisite for achieving sustainable seedling production in afforestation efforts. However, as storage duration increases, ageing processes in seeds accelerate viability loss through mechanisms such as membrane deterioration, reduced enzymatic activity, and decreased respiration efficiency. Recent studies have demonstrated that impairments in mitochondrial functions during long-term storage play a decisive role in the reduction of seed viability through increased reactive oxygen species production and oxidative stress. In addition, factors such as storage temperature and seed moisture content have been reported to exert significant effects on germination success and seedling development depending on species-specific responses (Edwards, 1998; Wawrzyniak et al., 2020; Małecka et al., 2021; Fuchs et al., 2023). The effects of long-term storage (10–30 years) on germination success in conifer seeds have been extensively investigated in species such as *Picea abies* (L.) Karst., *Picea glauca* (Moench) Voss, and *Picea mariana* (Mill.) BSP, and storage temperature (particularly 0–5 °C), seed moisture content, and the physiological condition prior to storage have been identified as the most critical factors influencing viability loss (Tomášková et al., 2014; Simpson et al., 2008; Suszka et al., 2005; Bonner, 1998; Simpson et al., 2004). Similarly, Edwards and Wang (1995) reported that conifer seeds can be stored at low temperatures for up to 25 years; however, germination rates tend to decline in a non-linear manner with increasing storage duration. Recent studies have further demonstrated that prolonged storage may induce oxidative stress and mitochondrial dysfunction, which are closely associated with reduced seed viability and deterioration of germination performance (Małecka et al., 2021).

The limited number of studies conducted directly on Oriental spruce indicate that long-term storage conditions significantly affect germination performance. Göktürk et al. (2019) demonstrated that seeds stored at 0–5 °C for nine years exhibited variation in germination rates depending on provenance, with some origins showing greater tolerance to storage-related stress. This finding suggests that germination responses in long-term stored Oriental spruce seeds are influenced not only by ageing-related physiological constraints but also by underlying genetic factors.

Various pre-treatment methods have been employed to improve germination performance in long-term stored seeds. These methods generally aim to enhance water uptake, weaken physical dormancy, stimulate metabolic activity, or optimize seed moisture conditions prior to germination. Common approaches include hot water soaking, short-term heat-shock applications, mechanical or chemical scarification, and controlled drying treatments (Wawrzyniak et al., 2020; Zembele & Ngulube, 2022). For instance, Himanen et al. (2013) reported that soaking in water can increase germination rate in *P. abies*, while Simak (1991) and Chen and Varis (1990) indicated that short-term heat treatments may contribute to dormancy breaking in certain conifer species. Therefore, evaluating the effects of pre-treatments on long-term stored *Picea* seeds is essential for understanding the impacts of physiological ageing and for improving seedling production strategies.

Provenance-based variation in seed germination is a well-documented phenomenon in forest tree species (Güney et al., 2014, 2015). Studies on *P. abies* have shown that seeds from different geographic origins exhibit consistent and predictable differences in germination onset, optimal temperature requirements, and germination rate (Dormling, 1979). This suggests that post-storage germination responses may also vary among Oriental spruce populations collected from different ecological zones. Accordingly, evaluating long-term storage and pre-treatment methods across a wide range of provenances representing the species' natural distribution is crucial for both understanding fundamental physiological processes and providing guidance for silvicultural practices. Recent studies further indicate that germination and early seedling performance are influenced not only by intrinsic seed properties but also by external applications and changing environmental conditions, and that climate change may alter regeneration dynamics in woody species (Sevik et al., 2025; Canturk et al., 2025).

In this context, the present study aims to determine how germination performance of Oriental spruce seeds changes following approximately 11 years of storage at 4–5 °C when subjected to different pre-treatment methods. By jointly evaluating (i) inter-population variation, (ii) the effects of pre-treatment types, and (iii) the potential physiological limitations associated with long-term storage, this study addresses an important gap in the existing literature.

Materials and methods

Study area and seed material

The seeds of Oriental spruce used in this study were obtained from 20 different provenances representing the natural distribution range of the species (Table 1). Cones were collected from five selected trees within each provenance, and samples were prepared at both population and individual levels. After extraction from the cones, the seeds were cleaned, moisture content was equilibrated, and the seeds were stored in sealed polyethylene bags under cold storage conditions at 0–4 °C. The seed material was maintained under these conditions for a total of 11 years until the initiation of the experiment. Natural range of Oriental spruce across Türkiye showing the locations of the sampled populations.

A completely randomized full-factorial experimental design consisting of 20 provenances × 5 trees × 3 experimental variants × 3 replicates was employed in this study. Each replicate consisted of 25 seeds. Experimental units were randomly arranged, and all experimental variants were conducted under standardized conditions. Prior to germination, two different pre-treatments were applied to the seeds. The treatment parameters were determined based on thermal application protocols commonly used for long-term stored conifer seeds (Üçler et al. 2018; Rawat & Uniyal, 2011).

1. Control (untreated): No physical or chemical pre-treatment was applied. Seeds were directly subjected to germination.
2. Hot water treatment (40 °C, 1 h): Seeds were placed in small cloth bags and immersed in a water bath at 40 °C for 60 minutes. The temperature was maintained constant throughout the treatment. Afterward, seeds were briefly placed on filter paper to remove surface moisture.
3. Dry heat treatment (90 °C, 1–1.5 min): Seeds, enclosed in thin paper packets, were exposed to dry heat in a laboratory oven set at 90 °C for 1–1.5 minutes. At the end of the treatment, seeds were immediately removed and allowed to cool at room temperature.

Table 1. Geographic characteristics of the sampled Oriental spruce populations

Pop no	Provenance	Elevation (m a.s.l.*)	Latitude (N)	Longitude (E)
1	Ordu-Çambaşı	1050–1350	40°43'20"	37°57'15"
2	Ordu-Çambaşı	1650–1950	40°38'58"	37°50'16"
3	Giresun-Dereli	1050–1350	40°35'50"	38°26'17"
4	Giresun-Dereli	1350–1650	40°33'48"	38°25'15"
5	Giresun-Dereli	1650–1950	40°33'45"	38°26'15"
6	Trabzon-Karadağ	1350–1650	40°56'37"	39°24'00"
7	Trabzon-Karadağ	1650–1950	40°55'38"	39°22'55"
8	Trabzon-Maçka	1350–1650	40°40'30"	39°25'45"
9	Trabzon-Maçka	1650–1950	40°40'16"	39°25'30"
10	Trabzon-Çaykara	1350–1650	40°34'24"	40°12'03"
11	Trabzon-Çaykara	1650–1950	40°34'30"	40°12'03"
12	Rize-İkizdere	1650–1950	40°38'50"	40°32'12"
13	Rize-Çamlıhemşin	1350–1650	40°52'28"	40°56'15"
14	Rize-Çamlıhemşin	1950–2250	40°48'40"	40°56'45"
15	Torul-Kürtün	1050–1350	40°35'10"	39°20'40"
16	Torul-Kürtün	1350–1650	40°34'30"	39°20'45"
17	Torul-Örümcek	1350–1650	40°39'30"	39°15'10"
18	Torul-Örümcek	1650–1950	40°39'45"	39°15'02"
19	Artvin-Atilla	1050–1350	41°07'23"	41°07'22"
20	Artvin-Taşlıca	1650–1950	41°08'00"	41°36'40"

* a.s.l.: above sea level.

Fig. 1. Natural range of Oriental spruce across Türkiye showing the locations of the sampled populations

Germination tests

Following pre-treatments, seeds were placed in Petri dishes containing double-layered moistened filter paper. Seeds were evenly spaced within each Petri dish, and excessive surface moisture was avoided. Germination was conducted in a growth chamber at 20 ± 1 °C under a 16 h light / 8 h dark photoperiod and approximately 70–75% relative humidity conditions, which are commonly recommended for germination studies in spruce species. Germination was defined as the emergence of a radicle at least 2 mm in length (ISTA, 2020). Germinated seeds were removed from the medium, and counts were recorded on days 7, 14, 21, 28, and 35. Germination percentage (GP) for each population and tree, depending on the experimental variant, was calculated based on the cumulative number of germinated seeds recorded on the specified observation days.

$$GP\ (\%) = (\sum n_i / N) \times 100$$

Here, n_i represents the number of seeds germinated at a given time point, and N denotes the total number of seeds.

Mean germination time (MGT) was calculated using the following formula.

$$MGT = \sum(n_i \times t_i) / \sum n_i$$

Here, n_i represents the number of seeds germinated on the ith counting day, t_i denotes the number of days until germination, and $\sum n_i$ indicates the total number of germinated seeds (Ranal & Santana, 2006).

Statistical analyses

The effects of provenance, variant groups, and their interactions on germination percentage and germination rate were evaluated using one-way and multifactor analysis of variance (ANOVA). Percentage data were subjected to arcsine square-root transformation when necessary to meet the assumption of normality. Differences among treatment means were compared using Duncan's multiple range test.

Principal Component Analysis (PCA) was additionally used to assess the multivariate relationships among populations according to germination characteristics. Hierarchical cluster analysis was also performed to evaluate similarities among populations based on germination characteristics. All analyses were performed based on population mean values, and the level of significance was set at $p < 0.05$.

Results

Effects of experimental variants on germination performance

The effects of pre-treatment applications on germination performance are presented in Table 2 and Figures 2 and 3. The applied variant groups resulted in significant differences in both final germination percentage and germination dynamics.

The hot water treatment (HotWater_40 °C) showed mean germination percentages comparable to the control group, but higher than those observed under the dry heat treatment (Table 2). In contrast, the short-term dry heat treatment (DryHeat_90 °C) was characterized by the lowest mean germination percentage and negatively affected germination success. Differences among variants were found to be statistically significant ($p < 0.05$).

In terms of germination rate, mean germination time (MGT) values also differed markedly among variants. MGT values in the control and hot water variants were very similar, indicating faster germination. Conversely, the DryHeat_90 °C variant resulted in higher MGT values, reflecting delayed germination. According to Duncan's multiple range test, the variants were separated into three statistically distinct groups for both GP and MGT. The distribution of germination percentage was more homogeneous under the hot water variant, whereas the dry heat variant was characterized by lower mean values and a wider range of variation (Fig. 2).

When these findings are considered together, it is evident that pre-treatment applications in long-term stored Oriental spruce seeds significantly influence not only the final germination percentage but also the rate of the germination process. In particular, the hot water treatment provides more balanced results in terms of faster germination dynamics, whereas the dry heat treatment appears to exacerbate physiological limitations following storage. The results of the two-way ANOVA showing the effects of population, variant, and their interaction on germination percentage (GP) and mean germination time (MGT) are presented in Table 3.

Table 2. Effects of variant groups on germination percentage (GP) and mean germination time (MGT) of Oriental spruce seeds

Pre-treatment	GP (%) ± SD	MGT (days) ± SD
Control	51.69 ± 1.51^{a}	11.57 ± 0.20^{a}
HotWater_40 °C	46.67 ± 1.65^{b}	11.67 ± 0.21^{b}
DryHeat_90 °C	39.17 ± 1.64^{c}	13.29 ± 0.24^{c}

Different letters within each column denote significant differences among variant groups (Duncan's test, $p < 0.05$).

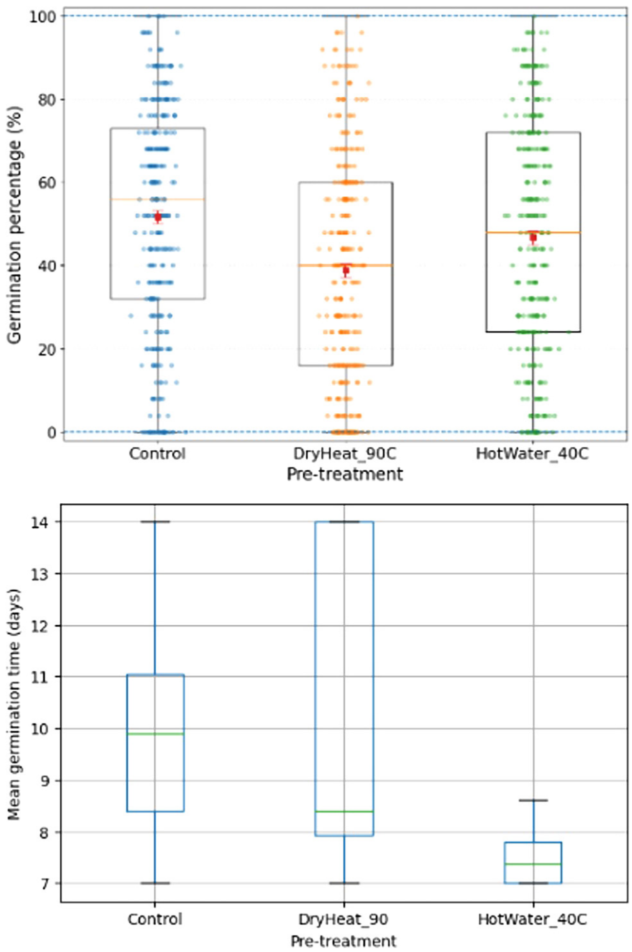

Fig. 2. Effects of variant groups on germination percentage (up) and mean germination time (down) of Oriental spruce seeds

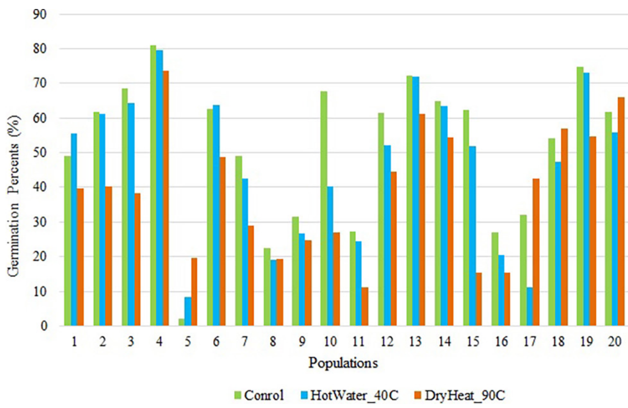

Fig. 3. Variation in germination percentages according to populations and variant groups

Table 3. Two-way ANOVA results for population and variant groups effects on GP and MGT of Oriental spruce

Source of variation	df	GP (p-value)	MGT (p-value)
Population	19	< 0.001	< 0.001
Pre-treatment	2	< 0.010	< 0.010
Population × Pre-treatment	38	< 0.050	< 0.050

The results of the two-way analysis of variance indicated that both germination percentage (GP) and mean germination time (MGT) in Oriental spruce seeds were significantly affected by provenance and pre-treatment factors. The provenance factor was highly significant for both variables ($p < 0.001$), demonstrating that even after long-term storage, germination capacity and germination dynamics are largely determined by provenance-specific characteristics. The pre-treatment factor also had a statistically significant effect on both GP and MGT ($p < 0.01$), indicating that the applied thermal variant groups influence not only final germination success but also the rate of the germination process. In addition, the significant provenance × pre-treatment interaction for both variables ($p < 0.05$) reveals that germination responses to pre-treatment applications vary among provenances (Table 3). The corresponding effect size (partial η^2) values for population, variant, and their interaction on GP and MGT are presented in Table 4.

Furthermore, effect size analyses revealed that the most dominant factor influencing both germination percentage (GP) and mean germination time (MGT) was provenance. The partial η^2 values calculated for the provenance factor indicated a large effect size for both variables, demonstrating that a substantial portion of the variation in germination traits is attributable to provenance-specific characteristics. While the pre-treatment factor exhibited a moderate effect size on GP and MGT, the effect size of the provenance × pre-treatment interaction ranged from small to moderate (Table 4). The limited variation in germination percentages associated with experimental variants across populations is also visually supported by the distribution patterns presented in Figure 3, confirming the relatively secondary influence of this factor compared to provenance.

As can be seen in Figure 3, although the changes associated with variant groups varied among populations, they generally followed a similar trend. The dry heat treatment (DryHeat_90 °C) resulted in higher mean germination percentages than the hot water treatment (HotWater_40 °C) in 15 populations and higher than the control in 3 populations.

The overall trend in germination indicates that GP values decreased with increasing elevation, with high-elevation populations exhibiting lower germination success. In contrast, the relationships between GP and latitude and longitude were relatively weak, suggesting that the effect of geographic location on germination success is secondary compared to elevation. Mean germination time (MGT) increased with elevation, indicating that germination occurred more slowly in high-elevation populations.

Germination performance of Oriental spruce seeds exhibited pronounced variation among populations (Table 5). Germination percentage (GP) ranged from approximately 10.04% to 78.04% across populations, indicating that provenance-specific differences in germination potential were maintained even under long-term storage conditions.

The highest mean germination percentage was recorded in the Dereli 1350–1650 m population (78.04%), followed by Kürtün 1050–1350 m (68.44%) and Örümcek 1350–1650 m (67.47%). In contrast, the Dereli 1650–1950 m population was clearly distinguished from the others by its very low germination percentage (10.04%), highlighting substantial differences in germination success among

Table 5. Germination percentage and germination rate values according to populations

Population	GP (%) ± SD	MGT (days) ± SD
Ordu 1050–1350	48.09 ± 3.92 defg*	9.91 ± 0.54
Ordu 1650–1950	54.49 ± 3.99 cde	10.77 ± 0.42
Dereli 1050–1350	57.07 ± 5.77 cd	10.12 ± 0.62
Dereli 1350–1650	78.04 ± 2.22 a	10.80 ± 0.49
Dereli 1650–1950	10.04 ± 1.96 ı	9.88 ± 0.66
Karadağ 1350–1650	20.27 ± 2.61 h	10.48 ± 0.63
Karadağ 1650–1950	27.64 ± 1.87 h	15.56 ± 0.63
Maçka 1350–1650	43.73 ± 3.66 fg	12.29 ± 0.53
Maçka 1650–1950	20.53 ± 2.40 h	13.21 ± 0.69
Çaykara 1350–1650	28.62 ± 3.39 h	13.38 ± 0.52
Çaykara 1650–1950	52.89 ± 2.49 cdef	13.13 ± 0.47
İkizdere 1650–1950	52.80 ± 3.00 cdef	12.03 ± 0.51
Çamlıhemşin 1350–1650	58.40 ± 3.69 bcd	13.53 ± 0.55
Çamlıhemşin 1950–2250	40.27 ± 2.79 g	11.75 ± 0.53
Kürtün 1050–1350	68.44 ± 3.20 b	13.45 ± 0.48
Kürtün 1350–1650	60.89 ± 3.24 bc	11.76 ± 0.39
Örümcek 1350–1650	67.47 ± 2.85 b	12.30 ± 0.61
Örümcek 1650–1950	61.51 ± 2.95 bc	13.16 ± 0.36
Artvin 1050–1350	44.98 ± 3.64 efg	11.41 ± 0.54
Artvin 1650–1950	20.09 ± 3.04 h	12.84 ± 0.58

* Different letters within the GP column indicate statistically significant differences among populations according to Duncan's multiple range test ($p < 0.05$).

Table 4. Effect sizes for population, variant groups, and their interaction on GP and MGT

Source of variation	GP (partial η^2)	Effect size	MGT (partial η^2)	Effect size
Population	> 0.40	large	> 0.35	large
Pre-treatment	~0.08–0.12	moderate	~0.07–0.10	moderate
Population × Pre-treatment	~0.05–0.07	small–moderate	~0.05–0.08	small–moderate

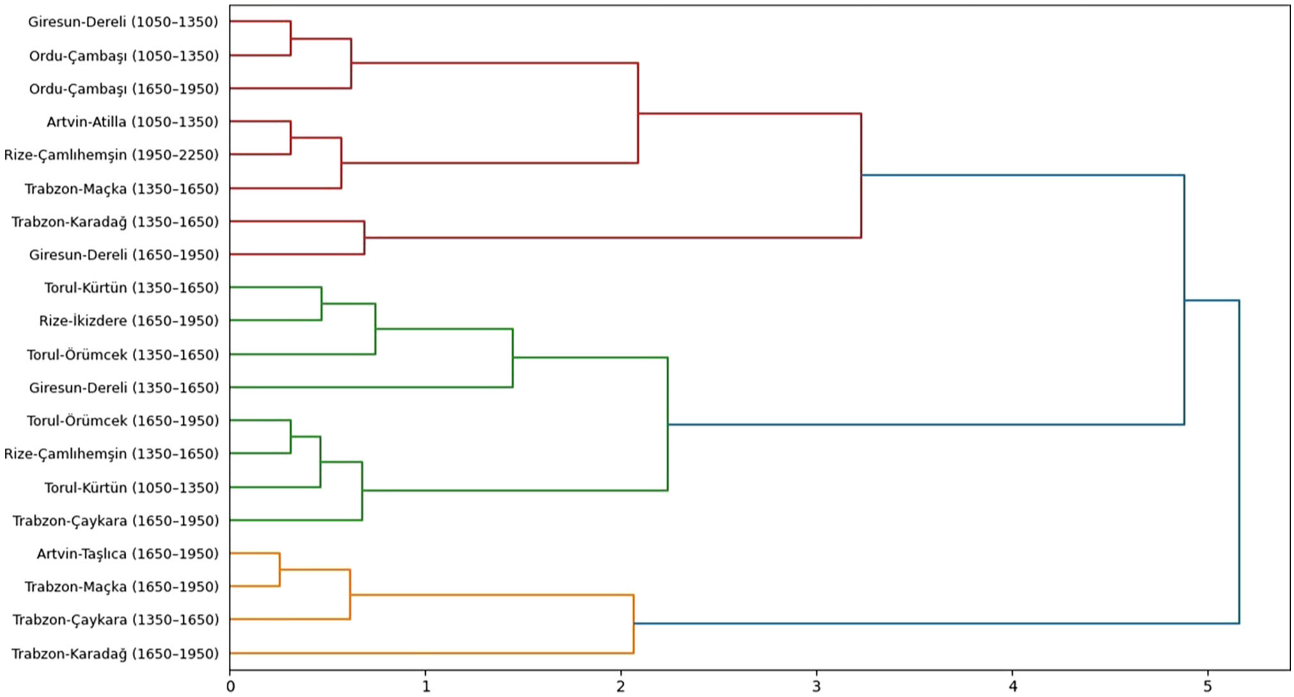

Fig. 4. Hierarchical cluster analysis based on GP and MGT values (dendrogram on the left)

populations. Different letters within the GP column indicate statistically significant differences among populations according to Duncan's multiple range test ($p < 0.05$).

Similarly, significant differences among populations were observed in terms of mean germination time. MGT values ranged from approximately 9.9 to 15.6 days. The lowest MGT values were recorded in Ordu 1050–1350 m (9.91 days) and Dereli 1650–1950 m (9.88 days), indicating faster germination in these populations. In contrast, the Karadağ 1650–1950 m population, which also exhibited low germination percentage, showed the highest MGT value (15.56 ± 0.63 days), indicating markedly delayed germination.

Populations located in close geographic proximity – such as Kürtün 1050–1350 m, Kürtün 1350–1650 m, Örümcek 1350–1650 m, and Örümcek 1650–1950 m – displayed similar values in both germination percentage (GP) and mean germination time (MGT). These populations were distinguished by relatively high GP values and consistent germination rates compared to others. To further reveal similarities among Oriental spruce populations in terms of germination performance, a hierarchical cluster analysis was conducted using GP and MGT variables (Fig. 4).

The results of the cluster analysis indicate that Oriental spruce populations do not exhibit a homogeneous structure in terms of germination percentage and germination rate, but rather are grouped into distinct performance clusters. Figure 5 shows the spatial distribution of the clustering groups formed according to GP and MGT values among the populations.

The populations were grouped under different clusters, and some populations within the same cluster were geographically close to each other. In contrast, some populations located in similar geographic regions were found in different clusters. The distribution of cluster groups revealed the presence of different germination performance patterns among populations (Fig. 5). The results of the principal component analysis (PCA) based on germination percentage (GP) and mean germination time (MGT) are presented in Figure 6.

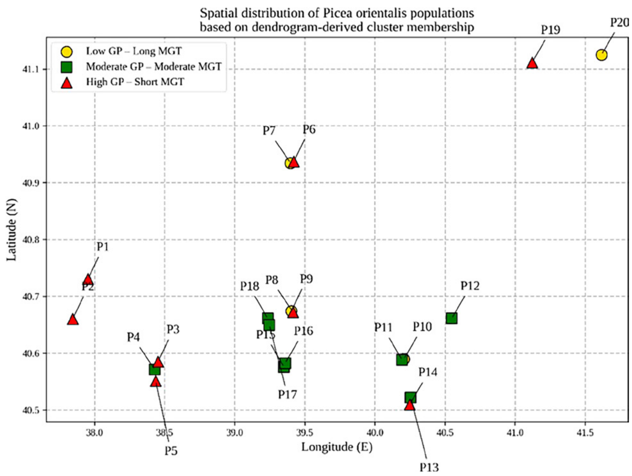

Fig. 5. Spatial distribution of hierarchical clustering groups based on GP and MGT values according to geographic location

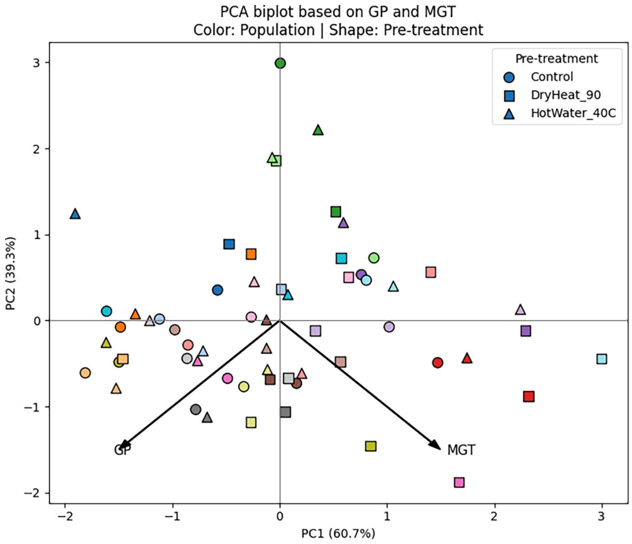

Fig. 6. PCA-Biplot based on GP and MGT

The first two principal components explained 100% of the total variance, with PC1 and PC2 accounting for 60.7% and 39.3% of the variance, respectively. The PCA biplot showed that the PC1 axis was associated with high GP and low MGT, whereas the PC2 axis reflected secondary variation.

Discussion

Germination performance also showed clear relationships with elevation and geographic distribution. Comparisons among populations from the same region but different elevation zones revealed distinct patterns. The pronounced variation in germination observed among populations in this study can be attributed to the high adaptive capacity of Oriental spruce within its natural distribution range. Studies by Turna (2004) have demonstrated that Oriental spruce populations in Türkiye exhibit clear ecological differentiation along watershed and elevation gradients, which directly influences seed morphology. The findings of the present study, particularly the lower germination success of high-elevation populations (e.g., Dereli 1650–1950 m), support the view of Donahue and Upton (1996) that variation is largely associated with climatic factors and elevation. The elevation-related differences identified in this study are also consistent with findings showing that karyotypic characteristics in Oriental spruce populations are positively correlated with elevation and that environmental factors play a decisive role in shaping chromosomal structures (İnceer et al., 2009). Turna (2004) further reported that neighboring populations with similar ecological characteristics tend to form comparable groups. Similarly, in the present study, populations from geographically close regions such as Kürtün and Örümcek exhibited similar germination strategies, indicating that, in addition to genetic structure, local adaptations play a key role in shaping post-storage seed performance.

Recent studies have demonstrated that environmental conditions, particularly differences related to temperature and water regime, shape germination behavior by influencing biochemical changes occurring within seeds. Kijowska-Oberc et al. (2023) reported that biochemical responses associated with drought stress vary depending on seed characteristics and stress duration, whereas Fang et al. (2017) demonstrated that germination traits in populations originating from different habitats are more strongly associated with environmental conditions than with phylogenetic relatedness. Similarly, Kijowska-Oberc et al. (2020) emphasized that germination capacity and seed physiological responses are shaped not only by genetic background and seed type, but also by the environmental conditions prevailing during seed development on the mother tree. The authors further indicated that changes in temperature and precipitation regimes may alter the physiological status of seeds by affecting biochemical processes such as proline accumulation and redox balance. In addition, seed germination has been described as a complex biophysical and biochemical process initiated by water uptake and involving enzyme activation, increased respiration, and mobilization of storage compounds (Liu et al., 2022; Welbaum et al., 1998). These findings suggest that, particularly in Oriental spruce populations developing across different elevation zones, physiological and biochemical differences occurring during seed development may represent one of the main factors underlying variation in germination performance.

The ability of seeds to maintain viability over a long period of 11 years is consistent with the orthodox seed characteristics of Oriental spruce; however, viability losses occurring during this process are a physiological reality. A study by Gu et al. (2024), which examined pine seeds (*Pinus densiflora*) stored at different temperatures for 20 years, demonstrated that storage temperatures such as +4 °C can lead to increased electrolyte leakage and deterioration of cell membrane integrity. This indicates that damage occurring during water uptake (imbibition) in aged seeds can inhibit germination. Similarly, recent experimental studies on tree species have shown that various environmental stresses and external applications can significantly alter physiological responses in plant tissues (Guney et al., 2023; Koç et al., 2026). On the other hand, Suszka et al. (2005) reported that Norway spruce (Picea abies) seeds can be stored for up to 29 years under appropriate moisture conditions, although a decline in germination capacity is inevitable. In the present study, the relatively low overall mean germination rate (45.84%) compared to fresh seeds can be attributed to the gradual depletion of internal biochemical reserves (e.g., carbohydrates) and the weakening of metabolic integrity over time.

Long-term storage studies on Norway spruce (Picea abies) seeds have demonstrated that seed viability and germination performance can be maintained for many years when appropriate moisture content and low-temperature conditions are ensured. Suszka et al. (2005) reported that P. abies seeds dried to low moisture content retained high germination percentages and stable mean germination times even after prolonged storage at low temperatures. This indicates that germination success is closely related not only to storage duration but also to the physiological characteristics and origin of the seeds. Similarly, it has been emphasized that seed resistance during storage and differences in germination rate may be associated with population-specific physiological variation (Roberts, 1973; Bonner, 1990). Barton (1961) highlighted that even under optimal storage conditions, biochemical processes associated with seed aging continue, ultimately leading to viability loss, and therefore seed longevity should be evaluated in conjunction with storage conditions. Walters et al. (2004) further demonstrated that declines in germination capacity can occur over time even at very low temperatures, suggesting that cumulative biological changes may affect viability even under long-term cold storage conditions in species such as Oriental spruce.

The success of ex situ seed storage in woody species is known to vary depending on species and provenance, and viability losses during long-term storage are considered inevitable (Wang et al., 1993). Atay et al. (1970) reported that germination percentages of Oriental spruce seeds decreased below 50% after 8 years of storage. In the present study, the results indicate that after 11 years of storage, approximately half of the provenances exhibited germination rates above 50%, while the other half remained below this threshold, with an overall mean of 45.84%. Even under low-temperature storage conditions, seeds continue to respire, making viability loss an expected outcome (Ürgenç & Odabaşı, 1971). Temperature has been identified as a key determinant of seed viability during long-term storage (Ürgenç, 1960; Nasreen et al., 2000). Similarly, long-term storage experiments on Picea glauca seeds have shown that seeds stored at +4 °C exhibit significant declines in both germination percentage and germination rate, whereas those stored at −20 °C germinate more rapidly and uniformly (Simpson et al., 2008). Based on the present findings, the observed changes in germination percentage and germination rate in seeds stored at 0–4 °C for 11 years appear to be influenced not only by storage temperature but also by storage duration and provenance characteristics.

It has previously been reported that germination percentage and germination rate in Oriental spruce seeds vary depending on storage duration, and that the extent of this variation differs among provenances. A general decline in germination capacity with increasing storage duration has also been observed, although some provenances appear to be more resistant to long-term storage than others (Göktürk et al., 2019). The differences in GP and MGT identified among populations in the present study indicate that germination performance is influenced not only by environmental or temporal factors but also by provenance-specific physiological and adaptive traits.

The effects of pre-treatments applied after long-term storage may also vary depending on seed origin. It has been reported that seeds derived from natural stands maintain more stable performance after pre-treatment compared to seed orchard material (Hilli et al., 2003). The fact that the Oriental spruce populations examined in this study were selected from natural stands may have influenced seed resistance after storage and their response to pre-treatments. In addition, pre-soaking treatments have been shown to enhance germination uniformity and improve nursery performance (Himanen & Nygren, 2014). Given that Oriental spruce is a relic and paleoendemic species under threat of genetic erosion (Yahyaoğlu et al., 1991), strategies for the storage and regeneration of its seeds are of critical importance. As also emphasized by Tomášková et al. (2014), seeds aged 10 years and older should still be considered highly valuable material for afforestation efforts, particularly in periods when high-quality fresh seeds are scarce.

When the results of the applied variant groups are evaluated, the dry heat treatment at 90 °C appears to have a relatively negative effect on seed viability and reduces germination success to a certain extent. This finding is consistent with the results of Moro et al. (2021), who reported that high-temperature heat shocks in woody species can eliminate germination and final viability. Although thermotolerance mechanisms exist in conifer seeds, Gifford and Taleisnik (1994) emphasized that temperatures above a certain threshold can disrupt cellular protein structures within the seed and halt metabolic activity. In contrast, the more balanced performance of the hot water treatment (40 °C) observed in this study is in line with the findings of Üçler et al. (2018), who identified moist heat treatment as the most effective pre-treatment method in subalpine Oriental spruce seeds. These results also agree with recent studies indicating that the effects of external applications on germination success depend on the type and intensity of the treatment as well as the sensitivity of the biological material (Sevik et al., 2025).

The findings further confirm that Oriental spruce seeds stored at 0–4 °C for as long as 11 years can still serve as a potential resource for afforestation efforts, particularly during periods when seed availability is limited. Tomášková et al. (2014) also noted that conifer seeds aged 10 years and older may remain usable despite quality loss. However, as emphasized by Göktürk et al. (2019), the fact that some provenances exhibit much greater resistance to storage stress than others highlights the critical importance of provenance selection in determining seed transfer zones and storage strategies.

The findings of this study clearly demonstrate that the primary factor shaping germination performance of Oriental spruce seeds after long-term storage is seed provenance. Genetic monitoring studies based on isoenzyme analyses have likewise revealed significant genetic differentiation among Oriental spruce populations, highlighting populations such as Torul–Örümcek as strategic reserves with high genetic diversity (Güney et al., 2019). The high effect size values calculated for germination percentage (GP) and mean germination time (MGT) further confirm that variation in these parameters largely originates from provenance-specific genetic and physiological differences. The relatively limited effect of the provenance × pre-treatment interaction indicates that no universal pre-treatment method is applicable to all materials; rather, each provenance exhibits a distinct sensitivity to applied treatments. This necessitates the development of tailored approaches that consider the biological limits of each provenance rather than relying on standardized protocols to improve germination success in aged Oriental spruce seeds. In addition, recent habitat modeling studies have shown that the distribution of woody species in Türkiye is sensitive to climatic variables and that suitable habitats may shift in the future. Therefore, provenance-based germination and storage studies are important not only for current production needs but also for future conservation and restoration planning (Canturk et al., 2025).

The results also indicate that germination success depends not only on the applied variants but also on the physiological condition of the seeds and storage duration. Recent studies have demonstrated that environmental conditions, particularly temperature and water regime, play a decisive role in seed viability and germination processes. Fang et al. (2017) reported that germination behavior in populations originating from different habitats is more strongly associated with environmental conditions than with phylogenetic relatedness, whereas Kijowska-Oberc et al. (2023) demonstrated that physiological responses associated with drought stress vary according to seed characteristics and stress duration. In addition, oxidative stress and mitochondrial alterations occurring under environmentally driven seed aging processes have been identified as important factors reducing seed viability and germination capacity (Małecka et al., 2021; Fuchs et al., 2023). These findings suggest that the variation observed in long-term stored Oriental spruce seeds may be influenced not only by storage duration itself but also by the environmental adaptation capacity of different populations. Indeed, the literature emphasizes that successful long-term storage strategies should take species- and provenance-specific physiological characteristics into consideration.

Conclusion

The findings of this study demonstrate that long-term stored Oriental spruce seeds can retain substantial germination potential depending on provenance and applied thermal variants. Oriental spruce is among the species under significant threat of genetic erosion due to its limited distribution range and relic–paleoendemic nature. The reduction of forest areas, improper utilization, and biotic disturbances pose a serious risk to the integrity of its gene pool. To mitigate these threats, the integration of ex situ (seed banking) and in situ conservation strategies is of critical importance for preserving the genetic structure shaped over thousands of years. The ability of long-term stored seeds to retain a certain level of germination capacity provides a significant advantage, as it allows the genetic material conserved in seed banks to be reintroduced into propagation programs or restoration efforts when needed.

References

Acarer A (2024) Role of climate change on Oriental spruce (*Picea orientalis* L.): Modeling and mapping. BioResources 19: 3845–3856. doi:10.15376/biores.19.2.3845-3856.

Atalay İ (1984) Regioning of the seed transfer of oriental spruce (*Picea orientalis* L.) in Turkey. Forest Tree Seed Breeding Institute Publ. No. 2, Ankara.

Atay İ, Ürgenç S & Odabaşı T (1970) Karaçam, Sarıçam ve Doğu Ladini tohumlarının 8 yıllık saklama deneme sonuçları, İÜ Orman Fakültesi Dergisi Seri A 20: 68–80.

Barton LV (1961) Seed preservation and longevity. Leonard Hill, London, UK.

Bonner FT (1990) Storage of seeds: potential and limitations for germplasm conservation. Forest Ecology and Management 35: 316–320. doi:10.1016/0378-1127(90)90230-9.

Bonner FT (1998) Seed longevity and dormancy of conifers stored at subfreezing temperatures: IU-

FRO Joint Publication: Seed Symposium ‘97: Advances in Tree Seed Research. Canadian Forest Service, Ottawa, ON, pp. 5–9.

Boydak M (1996) Variation in cone and seed characteristics among oriental spruce (*Picea orientalis* (L.) Link.) provenances. Istanbul University Journal of the Faculty of Forestry, Series A 46: 15–40.

Canturk U, Koç İ, Erdem R, Ozturk Pulatoglu A, Donmez S, Ozkazanc NK, Sevik H & Ozel HB (2025) Climate-driven shifts in wild cherry (*Prunus avium* L.) habitats in Türkiye: A multi-model projection for conservation planning. Forests 16: 148. doi:10.3390/f16091484.

Chen H & Varis S (1990) Hot-water treatment effects on the germination of conifer seeds: Proceedings of the IUFRO Symposium on Seed Quality of West Highland Species (ed. by DGW Edwards) Forestry Canada, Victoria, BC, pp. 24–35.

Donahue JK & Upton JL (1996) Geographic variation in leaf, cone and seed morphology of *Pinus greggii* in native forests. Forest Ecology and Management 82: 145–157. doi:10.1016/0378-1127(95)03677-6.

Dormling I (1979) Temperature requirements for the germination of *Picea abies* provenances. Holzforschung 33: 115–123.

Edwards DGW & Wang BSP (1995) Longevity of conifer seeds during long-term storage: Proceedings of the International Symposium on Forest Seed, IUFRO Publication, pp. 56–72.

Edwards DGW (1998) Seed aging, viability and membrane deterioration in conifers: Seed Biology and Technology: Applications and Advances (ed. by M Black & R Lin) Haworth Press, New York, pp. 145–163.

Fang X-W, Zhang J-J, Xu D-H, Pang J, Gao T-P, Zhang C-H, Li F-M & Turner NC (2017) Seed germination of Caragana species from different regions is strongly driven by environmental cues and not phylogenetic signals. Scientific Reports 7: 11248. doi:10.1038/s41598-017-11294-x.

Fuchs H, Małecka A, Budzińska A, Jarmuszkiewicz W, Ciszewska L, Staszak AM, Kijowska-Oberc J & Ratajczak E (2023) High-throughput method for Oxygen Consumption Rate measurement (OCR) in plant mitochondria. BMC Plant Biology 23: 496. doi:10.1186/s12870-023-04516-0.

Gifford DJ & Taleisnik E (1994) Heat-shock response of Pinus and Picea seedlings. Tree Physiology 14: 103–110. doi:10.1093/treephys/14.1.103.

Göktürk A, Solhan İ, Temel F & Ölmez Z (2019) Saklama süresinin Doğu ladini (*Picea orientalis* L.) tohumlarının çimlenme yüzdesi ve hızı üzerine etkisi. Bartın Orman Fakültesi Dergisi 21: 182–190. doi:10.24011/barofd.471183.

Gu DE, Han SH & Kang KS (2024) Viability and integrity of *Pinus densiflora* seeds stored for 20 years at three different temperatures. Conservation Physiology 12: coae046. doi:10.1093/conphys/coae046.

Guney D, Koç İ, Isinkaralar K & Erdem R (2023) Variation in Pb and Zn concentrations in different species of trees and shrubs and their organs depending on traffic density. Baltic Forestry 29: 141–152. doi:10.46490/BF661.

Güney D, Atar E, Atar F & Turna İ (2014) Effects of different pretreatments and seed collection dates of oriental hornbeam (*Carpinus orientalis* Mill.) seeds on germination. Fresenius environmental bulletin 23: 2554–2559.

Güney D, Atar F, Atar E, Turna İ & Kulaç Ş (2015) The effect of pre-treatments and seed collection time on the germination characteristics of common hornbeam (*Carpinus betulus*) seeds in the Eastern Black Sea Region, Turkey. Seed Science and Technology 43: 1–9. doi:10.15258/sst.2015.43.1.01.

Güney D, Yahyaoğlu Z, Bayraktar A, Atar F & Turna İ (2019) Genetic diversity of *Picea orientalis* (L.) Link populations in Turkey. Šumarski list 143: 539–547. doi:10.31298/sl.143.11-12.4.

Hilli A, Tillman-Sutela E & Kauppi A (2003) Germination of pretreated Scots pine (*Pinus sylvestris* L.) seeds after long-term storage. Canadian Journal of Forest Research 33: 47–53. doi:10.1139/x02-155.

Himanen K & Nygren M (2014) Effects of seed pre-soaking on the emergence and early growth of containerized Norway spruce seedlings. New Forests 45: 71–82. doi:10.1007/s11056-013-9392-6.

Himanen K, Lilja A, Rytkönen A & Nygren M (2013) Soaking effect on seed germination and fungal infection in *Picea abies*. Scandinavian Journal of Forest Research 28: 1–7. doi:10.1080/02827581.2012.683037.

ISTA (2020) International Rules for Seed Testing. International Seed Testing Association (ISTA), Zurich.

İnceer H, Güney D, Hayırlıoğlu-Ayaz S, Özcan M, Turna İ & Üçler AÖ (2009) Karyotype variation within some native populations of oriental spruce (*Picea orientalis*) in Turkey. Biologia 64: 1076–1084. doi:10.2478/s11756-009-0185-5.

Kijowska-Oberc J, Dylewski Ł & Ratajczak E (2023) Proline concentrations in seedlings of woody plants change with drought stress duration and are mediated by seed characteristics: A meta-analysis. Scientific Reports 13: 15157. doi:10.1038/s41598-023-40694-5.

Kijowska-Oberc J, Staszak AM, Wawrzyniak MK & Ratajczak E (2020) Changes in proline levels during seed development of orthodox and recalcitrant seeds of genus Acer in a climate change scenario. Forests 11: 1362. doi:10.3390/f11121362.

Koç İ, Sevik H, Nechita C, Canturk U, Iordache AM & Camarero JJ (2026) Selenium bioaccumulation in industrialized urban forests: case study of five tree species used for phytoremediation. Urban Ecosystems 29: 73. doi:10.1007/s11252-026-01935-3.

Liu S, Wang W, Lu H, Shu Q, Zhang Y & Chen Q (2022) New perspectives on physiological, biochemical and bioactive components during germination of edible seeds: A review. Trends in Food Science & Technology 123: 187–197. doi:10.1016/j.tifs.2022.02.029.

Małecka A, Ciszewska L, Staszak A & Ratajczak E (2021) Relationship between mitochondrial changes and seed aging as a limitation of viability for the storage of beech seed (*Fagus sylvatica* L.). PeerJ 9: e10569. doi:10.7717/peerj.10569.

Moro AVI, Bravo SJ, Abdala NR, Borghetti F, Chaib AM & Galetto L (2021) Heat shock effects on germination and seed survival of five woody species from the Chaco region. Flora 275: 151751. doi:10.1016/j.flora.2020.151751.

Nasreen S, Khan BR & Mohmad AS (2000) The effect of storage temperature, storage period and seed moisture content on seed viability of soya bean. Pakistan Journal of Biological Science 3: 2003–2004. doi:10.3923/pjbs.2000.2003.2004.

Özpay M & Boydak M (1995) Dormancy and germination characteristics of *Picea orientalis* seeds. İstanbul Üniversitesi Orman Fakültesi Dergisi, Seri A 45: 49–65.

Ranal MA & Santana DG (2006) How and why to measure the germination process? Brazilian Journal of Botany 29: 1–11. doi:10.1590/S0100-84042006000100002.

Rawat BS & Uniyal AK (2011) Variability in cone and seed characteristics and seed testing in various provenances of Himalayan spruce (*Picea smithiana*). Journal of Forestry Research 22: 603–610. doi:10.1007/s11676-011-0203-7.

Roberts EH (1973) Predicting the storage life of seeds. Seed Science and Technology 1: 449–514.

Sevik H, Ucun Ozel H, Yildiz Y & Ozel HB (2025) Effects of adding Fe2O3 and Fe3O4 nanoparticles to soil on germination and seedling characteristics of Oriental beech. BioResources 20: 70–82.

Simak M (1991) Heat-shock treatments and viability of spruce seeds: Proceedings of the IUFRO Symposium on Tree Seed Problems, Victoria, BC, pp. 115–124.

Simpson JD, Wang BSP & Daigle BI (2004) Long-term seed storage of various Canadian hardwoods and conifers. Seed Science and Technology 32: 561–572.

Simpson JD, Daigle BI & Wang BSP (2008) Impact of storage on viability of white spruce seed. Tree Planters' Notes 53: 4–8.

Suszka B, Chmielarz P & Walkenhorst R (2005) How long can seeds of Norway spruce (*Picea abies* (L.) Karst.) be stored? Annals of Forest Science 62: 73–78. doi:10.1051/forest:2004082.

Tomášková I, Vítámvás J & Korecký J (2014) Testing of germination of spruce, pine and larch seed after 10 years from collection. Journal of Forest Science 60: 540–543. doi:10.17221/74/2013-JFS.

Turna İ (2004) Variation of morphological characters of Oriental spruce (*Picea orientalis*) in Turkey. Biologia, Bratislava 59/4: 519 – 526.

Üçler AÖ, Acar C, Yücesan Z & Oktan E (2018) Effect of thermal pretreatment on germination of seeds from different provenances of subalpine oriental spruce (*Picea orientalis* (L.) Link.) forest in Turkey. Journal of Sustainable Forestry 37: 302–315.

Ürgenç S & Odabaşı T (1971) Kızılçam (*Pinus brutia* Ten) tohumlarının uzun süreli (7yıl) kozalak içinde saklanmasının diğer saklama metodlarıyla mukayeseli sonuçları, İÜ orman Fakültesi Dergisi, Seri A 21: 82–92. doi:10.17099/jffiu.63213.

Ürgenç S (1960) Doğu Ladininde (*Picea orientalis* (L.) Link.) kozalak ve tohum üzerine araştırmalar. İstanbul Üniversitesi Orman Fakültesi Dergisi 10: 68–97. doi:10.17099/jffiu.15053.

Walters C, Wheeler L & Stanwood PC (2004) Longevity of cryogenically stored seeds. Cryobiology 48: 229–244. doi:10.1016/j.cryobiol.2004.01.007.

Wang BSP, Charest PJ & Downie B (1993) Ex situ storage of seeds of woody species. Forestry Chronicle 69: 189–199.

Wawrzyniak MK, Michalak M & Chmielarz P (2020) Effect of different conditions of storage on seed viability and seedling growth of six European wild fruit woody plants. Annals of Forest Science 77: 58. doi:10.1007/s13595-020-00963-z.

Yahyaoğlu Z & Genç M (2007) Fidan standardizasyonu: Standart Fidan Yetiştirmenin Biyolojik ve Teknik Esasları. Isparta: Süleyman Demirel Üniversitesi Yayınları, No: 75.

Yahyaoğlu Z, Demirci A & Genç M (1991) Relikt (Paleoendemik) Bir Tür, Doğu Ladini (*Picea orientalis* (L.) Link.). 1. Uluslararası Çevre Koruma Sempozyumu Bildirileri, 2. Cilt, İzmir, pp. 769–788

Zembele ER & Ngulube ES (2022) Effect of seed pretreatment methods on germination and early seedling growth of *Senna spectabilis*. International Journal of Forestry Research 2022: 6731479. doi:10.1155/2022/6731479.