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Research Article

Seed priming of *Prunus scoparia* through scarification and chemical treatments

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ABSTRACT- This study examined the effects of various pre-germination treatments on *Prunus scoparia* seeds collected from Zarand City in late summer 2024. The treatments aimed to overcome both mechanical and physiological dormancy. The seed coats were removed, and seeds were disinfected in a 1% sodium hypochlorite solution for 15–20 minutes, followed by rinsing with sterile water. A factorial experiment was conducted based on a completely randomized design with three replications. Four factors were evaluated: scarification (control and sulfuric acid for 10 minutes), stratification (control, moist chilling at +4 °C, and dry chilling at −20 °C), potassium nitrate (0%, 1%, and 2% potassium nitrate), and gibberellic acid (0 and 200 ppm gibberellic acid). Primary evaluations of vital indicators such as seed germination and shoot length of plantlets revealed that seed scarification with sulfuric acid, moist chilling at +4 °C, and treatment with 200 ppm gibberellic acid significantly enhanced germination performance. The results indicated that the combination of mechanical scarification, moist chilling, and gibberellic acid treatment effectively overcame dormancy barriers in *P. scoparia* seeds. This study highlights the importance of tailored pre-germination treatments to improve seed viability and subsequent plant establishment.

INTRODUCTION

Prunus scoparia, commonly known as the wild almond, is a xerophytic shrub predominantly found in the Zagros forests of Iran, with additional distribution across Turkey, Turkmenistan, and Afghanistan. It typically grows as a deciduous shrub or small tree, reaching heights of about 1–2 meters in most accessions, though some reports indicate it can attain 3–6 meters under optimal conditions (Gharaghani and Eshghi, 2014). Native to Iran, *P. scoparia* is characterized by its green shoots and remarkable ability to thrive in arid and semi-arid environments, demonstrating strong resistance to abiotic stresses such as drought and salinity. The species features a dense green canopy and long-lasting green branches that continue photosynthesis even after leaf drop in early summer. The leaves, generally green, fall early in the season, allowing the branches to maintain photosynthetic activity and carbohydrate production throughout the growing period. *P. scoparia* produces attractive flowers and exhibits a prolonged flowering period. The fruit stalk length ranges from 1.85 to 5.53 mm, while nut dimensions vary between 9.72–22.87 mm in length and 5.81–15.54 mm in width (Gharaghani and Eshghi, 2014). In addition to its morphological traits, *P. scoparia* plays a significant ecological role in stabilizing soil and controlling erosion, making it a valuable resource for landscape management and environmental restoration. Its adaptability and growth characteristics make it a promising candidate for breeding programs and reforestation efforts (Khadivi-Khub and Anjam, 2014). Due to its high drought resistance and ability to grow in infertile soils, *P. scoparia* is often used as a rootstock for domesticated almonds, particularly in arid and semi-arid regions (Hanelt, 2001). Its resilience to water scarcity and adaptability to nutrient-poor conditions further enhance its suitability as a rootstock for grafting cultivated almond varieties. The species also contributes to ecosystem stability through gum and resin production, which serve as natural defense mechanisms against pests and diseases (Gharaghani and Eshghi, 2014). In rural regions of Iran, the seeds are consumed as a source of high-quality protein, adding nutritional and economic value to the species. The gum production not only provides pest resistance but also supports ecological balance in its native habitats. *P. scoparia* exhibits substantial genetic variation among populations, influenced by environmental factors such as climate and soil conditions (Gharaghani and Eshghi, 2014). This diversity offers valuable potential for breeding programs aimed at improving cultivated almonds by introducing stress tolerance and hardiness traits. The species reproduces through both sexual and vegetative means, involving wind pollination, fruit development, and seed dispersal. It can also be propagated through cuttings, which allows for faster establishment of new plants

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without the delays associated with seed germination (Nezami and Gallego, 2023).

P. scoparia exhibits significant seed dormancy, which presents considerable challenges for germination and seedling establishment. The dormancy can be broadly categorized into two main types: physical and physiological dormancy. Physical dormancy is primarily caused by the hard-outer seed coat (epicarp), which restricts the penetration of water and oxygen necessary for germination. This mechanical barrier prevents the embryo from effectively absorbing moisture, leading to low germination rates (Labdelli et al., 2019). Physiological dormancy, on the other hand, is associated with the embryo itself and can be influenced by environmental conditions during seed maturation and harvesting. Abscisic acid (ABA), a hormone known to inhibit germination, plays a key role in maintaining this type of dormancy (Brahim et al., 2019). To improve germination rates, several treatments have been developed to overcome these dormancy mechanisms. One of the most effective approaches involves exposing seeds to cold temperatures, typically around 4 °C, for a specific period. This cold stratification mimics natural winter conditions, helping to break dormancy and promote germination (Bassouya et al., 2024). Mechanical scarification, such as gently abrading the seed coat with sandpaper, can also enhance water uptake by physically weakening the impermeable outer layer (Brahim et al., 2019). Among chemical treatments, gibberellic acid (GA_3) is widely recognized for its effectiveness in breaking seed dormancy. Studies have shown that soaking wild almond seeds in GA_3 solutions at concentrations ranging from 750 to 1500 ppm significantly increases germination percentages, especially when combined with cold stratification. For instance, six weeks of cold stratification followed by GA_3 treatment at 1500 ppm has been reported to yield the highest germination rates of up to 95.6% (Rahemi et al., 2011). Soaking seeds in solutions such as GA_3 or sulfuric acid can thus effectively reduce dormancy, with GA_3 being particularly effective in counteracting the inhibitory effects of ABA. Although less common, thermal treatments, subjecting seeds to controlled heat, can also aid in dormancy alleviation. Nevertheless, mechanical and chemical methods remain the most widely adopted and effective. Temperature plays a key role in dormancy and germination. Cold stratification at around 5 °C has proven particularly effective for wild almond seeds, as it simulates winter conditions that naturally promote dormancy release. The duration of stratification is equally important; periods of 4 to 8 weeks are generally optimal for enhancing germination, particularly when used in combination with hormonal treatments such as GA_3 or KNO_3 (Zeinalabedini, 2009). The dormancy of *P. scoparia* seeds significantly limits their germination potential, necessitating specific pretreatments to achieve successful seedling production. Understanding and addressing these dormancy mechanisms are essential for effective conservation and cultivation practices of this economically important species (Bassouya et al., 2024). Enhancing seed germination and seedling establishment not only supports the conservation of *P. scoparia* populations but also contributes to sustainable agricultural practices and environmental rehabilitation in arid and semi-arid regions. Improved germination techniques can further bolster reforestation initiatives by increasing the survival rates of seedlings in challenging environments. The aim of this experiment was to identify and evaluate effective pre-germination treatments to overcome the physical and physiological dormancy of *Prunus scoparia* seeds. By comparing mechanical, chemical, and cold stratification methods, the study sought to improve germination rates and seedling establishment, thereby supporting the species' use in reforestation, breeding programs, and ecological restoration in arid and semi-arid regions.

MATERIALS AND METHODS

P. scoparia seeds were collected in late summer, specifically around the year 2024, from the Zarand region of Iran, located at approximately 30°48'57"N latitude and 56°33'57"E longitude. After peeling, the seeds of *P. scoparia* underwent a surface disinfection process to eliminate potential pathogens and contaminants. A sodium hypochlorite solution containing 1% active chlorine was prepared, as this concentration effectively disinfects seeds without causing significant damage to the seed tissues. The peeled seeds were immersed in the sodium hypochlorite solution for 15 to 20 minutes, and after the disinfection period, they were thoroughly washed three times with distilled water. This washing step was essential to remove any residual sodium hypochlorite solution, ensuring that no harmful chemicals remained on the seed surface that could adversely affect germination or seedling health. Various treatments were then applied to break both the mechanical dormancy caused by the seed coat and the deep physiological dormancy of the embryo, with the aim of increasing the germination percentage, germination speed, and initial growth of *P. scoparia* seedlings. A factorial experiment based on a completely randomized basic design (CRBD) was employed to systematically examine the effects of different treatments on seed germination and seedling growth, with three replications for each treatment combination. Considering four factors and various levels for each, a total of 36 experimental treatments were established. The study investigated multiple dormancy-breaking treatments to improve germination rates and early seedling development in *P. scoparia* seeds. These treatments included two levels of scarification (no scarification and scarification using 98% sulfuric acid for 10 minutes), three levels of chilling (no moist chilling, cold-moist stratification at 4 °C for two months, and dry chilling at -20 °C for two months), seed pretreatment in potassium nitrate solution (KNO_3) at three levels (0%, 1% solution, and 2% solution with seeds immersed for 22 hours), and seed pretreatment in GA_3 at two levels (0 and 200 mg/L solution with seeds immersed for 24 hours). After applying the experimental treatments, 25 seeds for each replication, totaling 75 seeds per treatment, were placed in plastic containers filled with a growing medium composed of wind-blown sand, soil, and perlite in a 1:1:1 ratio. The containers were transferred to a greenhouse maintained at a temperature of 20 ± 5 °C. The number of germinated seeds in each experimental unit was counted daily until no new germination occurred for three consecutive days, which indicated that the germination

process had effectively ceased. After 30 days, the total number of germinated seeds in each experimental unit was recorded to evaluate the effectiveness of each treatment in promoting seed germination and initial seedling growth.

The formula to calculate the final germination percentage (G%) was as follows:

$G\% = (\frac{Ng}{Nt})\times 100$ Eq. (1)

Ng = Number of germinated seeds

Nt = Total number of seeds planted in each experimental unit

The germination rate can be calculated using various methods. One common approach happens through the following equation:

$GR = \frac{Ng}{T}$ Eq. (2)

GR = Germination rate

Ng = Total number of seeds that germinated

T = Total time (in days) for which germination was observed

Only healthy and normal seedlings were selected for measurement to ensure that the data accurately reflect the growth potential of the plants. A random selection of seedlings from each experimental unit was made to avoid bias and ensure a representative sample. A standard ruler was used for measuring stem length. Average stem lengths from different experimental units were compared to assess how various treatments influenced initial seedling growth. The analysis was conducted using SAS software to determine which specific treatment and mean values were significantly different from one another. Duncan's multiple range test was employed.

RESULTS AND DISCUSSION

The results indicated that the different treatments had a significant effect on the germination percentage of wild almond seeds. Significant treatments were scarification, moist chilling, gibberellic acid (GA_3), the interaction between scarification and moist chilling, and the interaction between KNO_3 and gibberellic acid ($P < 0.01$). Also, potassium nitrate, the interaction between scarification and potassium nitrate, and the interaction between scarification and gibberellic acid were significant ($P < 0.05$). These findings showed that individual treatments and their interactions play an important role in enhancing the germination percentage of wild almond seeds (Table 1).

The findings emphasize the crucial role of specific treatment combinations in improving seed germination of wild almond. The optimal treatment, combining scarification, moist chilling, gibberellic acid, and potassium nitrate, produced the highest germination rate (94.66%). In contrast, treatments involving dry chilling, even when combined with other factors, resulted in markedly lower germination percentages (13.33%) (Table 2). A significant difference was also observed between the 0% and 2% potassium nitrate treatments, suggesting that higher concentrations may further inhibit germination (Table 3).

Table 1. Mean comparison obtained from variance analysis of measured traits

S.O.V	df	Mean square		
		Germination percentage (%)	Germination rate (day)	Seedling length (cm)
Scarification	1	22466.22**	25.42**	12.262**
Moist chilling	2	13952.33**	28.65**	19.32**
Potassium nitrate	2	343.22*	0.05	4.95**
Gibberellic acid	1	1856.32**	1.74**	16.62**
Scarification * Moist chilling	2	2279.88**	9.55**	0.72
Scarification * Potassium nitrate	2	309.88*	0.092	1.13
Scarification * Gibberellic acid	1	765.00*	1.02**	13.78**
Moist chilling * Potassium nitrate	4	81.66	0.54	0.30
Moist chilling * Gibberellic acid	2	176.39	0.12	2.97**
Potassium nitrate * Gibberellic acid	2	1056.39**	0.22*	2.63**
Scarification * Moist chilling * Potassium nitrate	4	170.32	0.036	0.52
Scarification * Moist chilling * Gibberellic acid	2	5.68	0.055	0.39
Scarification * Potassium nitrate * Gibberellic acid	4	41.33	0.016	1.02
Moist chilling * Potassium nitrate * Gibberellic acid	4	208.33	0.042	0.39
Scarification * Moist chilling * Gibberellic acid * Potassium nitrate	4	168.55	0.013	1.1*
Error	72	99.44	0.063	0.48
CV (%)		19.44	27.66	13.12

ns, ** Insignificant and significant at $P < 0.01$, respectively.

Table 2. Effects of applied treatments on germination percentage (%), germination rate (day), and seedling length (cm) of wild almond seeds

				Treatment		
				Germination (%)	**Germination rate (day)**	**Seedling length (cm)**
No scarification	C (25 °C)	N (0%)	GA (0)	19.33	0.0.	4.7
			GA (200)	44.69	0.5	4
		N (1%)	GA (0)	28.14	0.4	4.6
			GA (200)	28.96	0.2	4
		N (2%)	GA (0)	31.33	0.4	3.8
			GA (200)	25.39	0.1	4.3
	C (4 °C)	N (0%)	GA (0)	56.23	1.1	5.1
			GA (200)	53.39	1.2	5.9
		N (1%)	GA (0)	60.36	1.3	5.3
			GA (200)	59.89	1.4	5.5
		N (2%)	GA (0)	43.16	0.7	5
			GA (200)	44.23	0.7	5.1
	C (-20 °C)	N (0%)	GA (0)	13.33	0.03	3.8
			GA (200)	38.19	0.5	4.8
		N (1%)	GA (0)	29.16	0.3	4.2
			GA (200)	18.15	0.03	2.5
		N (2%)	GA (0)	18.33	0.2	3.5
			GA (200)	19.36	0.2	4.1
Scarification	C (25 °C)	N (0%)	GA (0)	30.28	0.3	4
			GA (200)	68.39	0.7	6.8
		N (1%)	GA (0)	37.44	0.4	4.2
			GA (200)	35.63	0.5	4
		N (2%)	GA (0)	33.96	0.3	4.2
			GA (200)	52.14	0.6	4.5
	C (4 °C)	N (0%)	GA (0)	83.25	2.7	5
			GA (200)	94.66	3.3	7.9
		N (1%)	GA (0)	89.36	2.6	4.8
			GA (200)	92.14	3.2	6.5
		N (2%)	GA (0)	92.23	2.8	4.8
			GA (200)	93.16	3	6.8
	C (-20 °C)	N (0%)	GA (0)	51.39	0.5	4.8
			GA (200)	78.96	1	6
		N (1%)	GA (0)	27.67	0.8	4
			GA (200)	67.29	0.9	4.8
		N (2%)	GA (0)	59.74	0.5	3.9
			GA (200)	70.28	1	5

Scarification, no scarification, no moist chilling (25 °C), moist chilling (4 °C), dry chilling (-20 °C), and KNO_3 (N).

Table 3. Mean comparison from the main effects of experimental factors

Treatment	**Level**	**Average**		
		Germination percentage (%)	**Germination rate (day)**	**Seedling length (Cm)**
Scarification	Control	33.93^{b}	0.37^{b}	4.4^{b}
	98%	61.44^{a}	1.44^{a}	5.06^{a}
Moist chilling	Control	32.98^{c}	0.22^{c}	4.44^{b}
	4 °C	70.11^{ab}	1.88^{a}	5.66^{a}
	-20 °C	40^{b}	0.44^{b}	4.22^{b}
Potassium nitrate	0	51.10^{a}	0.90^{b}	4.15^{a}
	1%	46.77^{ab}	0.84^{a}	4.39^{b}
	2%	45.33^{b}	0.88^{b}	4.69^{b}
Gibberellic acid	0	44.52^{b}	0.74^{b}	4.32^{b}
	200 mg/L	52.63^{a}	1^{a}	5.33^{a}

The interaction between scarification and chilling had a highly significant effect (at the 1% level) on germination speed. Similarly, the interaction between scarification and gibberellic acid was also significant at the 1% level. The interaction between potassium nitrate and gibberellic acid showed significance at the 5% level. These findings suggest that while potassium nitrate alone may reduce germination rates, its combination with gibberellic acid can modify this effect (Table 1). The highest germination rate observed was 3.3 germinated seeds per day, achieved

through the interaction of three key factors: scarification, moist chilling, and gibberellic acid (200 mg/L). Conversely, the lowest germination rate (0.03 germinated seeds per day) resulted from the combination of dry chilling, potassium nitrate (1%), and gibberellic acid (200 mg/L). These results demonstrate that the combination of scarification, moist chilling, and gibberellic acid is particularly effective, whereas certain treatments involving dry chilling and potassium nitrate can severely hinder germination (Table 2). Overall, the results indicate that seed scarification with sulfuric acid, moist chilling, and gibberellic acid at 200 mg/L are effective methods for increasing the germination rate of wild almond seeds. In contrast, potassium nitrate does not contribute positively to germination under these conditions (Table 2). The interaction between scarification and gibberellic acid also had a significant effect at the 1% level, indicating that combining these two treatments can synergistically enhance stem length more than either treatment alone. The interaction between chilling and gibberellic acid was similarly significant at the 1% level, while the interaction between potassium nitrate and gibberellic acid showed significance at the 1% level as well. This highlights the importance of nutrient availability in conjunction with growth hormones for optimal stem elongation. Moreover, the interaction among all four factors (scarification × chilling × potassium nitrate × gibberellic acid) showed a significant effect at the 5% level. This complex interaction suggests that combined treatments can produce greater effects on stem length than individual treatments alone, emphasizing the need for an integrated approach to treatment optimization (Table 1). The study of stem length revealed significant differences among treatment combinations. The maximum stem length (7.9 cm) was achieved through the interaction of scarification, moist chilling, and gibberellic acid (200 mg/L). In contrast, the combination of dry chilling, 1% potassium nitrate, and gibberellic acid resulted in reduced seedling growth (2.5 cm) (Table 2). Overall, seed scarification with sulfuric acid, moist chilling, zero potassium nitrate, and gibberellic acid at 200 mg/L were the most effective treatments for enhancing shoot length in wild almond seeds (Table 3).

Seed dormancy is an essential survival strategy that enhances the resilience of plants in natural environments. By allowing seeds to remain inactive until conditions become favorable, this mechanism ensures successful germination and establishment of seedlings, thereby supporting species persistence and ecosystem stability. Understanding seed dormancy is therefore crucial for both conservation initiatives and agricultural practices aimed at improving crop yields and maintaining plant biodiversity (Postma and Ågren, 2022). Seed scarification with sulfuric acid significantly increased germination percentage, likely by breaking down the hard seed coat and facilitating water uptake. Moist chilling also resulted in a marked improvement in germination, possibly by simulating the natural winter conditions required for dormancy release. Similarly, gibberellic acid (200 mg/L) markedly enhanced germination rates. In contrast, potassium nitrate, compared with the control (no potassium nitrate), was found to reduce the germination percentage of wild almond seeds. Combining cold stratification with other treatments, such as gibberellic acid or scarification, can further enhance germination performance. For instance, soaking seeds in gibberellic acid following cold stratification has been shown to produce better germination outcomes than either treatment alone (Rahemi et al., 2011).

The concentration of KNO_3 plays a key role in seed dormancy breaking and germination. For instance, a concentration of 250 mg/L has been identified as optimal for certain species, resulting in significant increases in both germination percentage and rate. In studies involving other species, lower concentrations (0.25 mM) were associated with higher germination rates, whereas higher concentrations tended to delay germination (Hernández et al., 2021; Duermeyer et al., 2018). Potassium nitrate positively influences dormancy breaking and germination in wild almond seeds through its effects on water uptake and hormonal regulation. Optimal concentrations, particularly when combined with cold stratification, can markedly enhance germination performance. Sulfuric acid scarification effectively weakens the hard seed coat of wild almond seeds, which otherwise acts as a major barrier to water absorption and gas exchange. This treatment enables seeds to overcome physical dormancy, leading to improved germination rates (Falah et al., 2014). Research further indicates that longer exposure times to sulfuric acid correlate with higher germination percentages. For example, treatments lasting up to four hours have shown substantial increases in germination, with some studies reporting up to 90% germination following optimal treatment durations. Sulfuric acid scarification has been found more effective than other dormancy-breaking methods in various species, including wild almonds. It outperforms both mechanical scarification and alternative chemical treatments in terms of germination percentage and rate (Al-Hadedy et al., 2024). Cold stratification also plays an essential role in enhancing germination. An optimal stratification period of 8–10 weeks has been reported to significantly increase germination percentages in some almond cultivars, while for wild almonds, stratification periods of 30–50 days have proven effective in promoting germination (Zeinalabedini et al., 2009).

Scarification of wild almond seeds with sulfuric acid is an effective method for enhancing germination by breaking seed dormancy. This treatment significantly increases both the germination percentage and the speed of germination, making it a valuable technique in agricultural practices aimed at improving seed viability and establishment. GA_3 has also been shown to effectively break dormancy in wild almond seeds. Studies indicate that soaking seeds in GA_3 solutions significantly increases germination percentages. For instance, concentrations of 750, 1000, or even 1500 ppm of GA_3 have produced notable improvements in germination rates when combined with cold stratification treatments (Abou Rayya et al., 2021). Another study reported that a concentration of 125 ppm resulted in the highest germination percentage (83.3%) at a lower temperature (7 °C), while higher concentrations (up to

500 ppm) also had positive effects, but were less efficient than the lower concentration at that temperature (Rouhi et al., 2005). Temperature plays a key role in determining the effectiveness of GA_3 treatments. Lower temperatures (around 7 °C) have reportedly enhanced the stimulatory effects of GA_3 on germination compared to higher temperatures (22 °C), demonstrating how crucial environmental conditions are for optimizing germination (Rouhi et al., 2005). Gibberellic acid significantly affects wild almond seed dormancy by promoting germination, optimal growth regulator concentrations and synergistic interactions with cold stratification. Specific responses depend on both the concentration and the environmental conditions, highlighting the importance of tailoring treatments to achieve effective seed germination and successful seedling establishment. A study on wild almond seedlings (*Prunus scoparia*) investigated the effects of various treatments on seed germination and seedling growth. The treatments included scarification, chilling, and gibberellic acid, all of which were found to significantly enhance seedling growth. In contrast, potassium nitrate did not show a significant effect on either the percentage or speed of germination, nor on seedling length, suggesting that its use is not beneficial in this context. Thus, scarification, cold stratification, and gibberellic acid together proved to be highly effective in increasing both the percentage and rate of germination in wild almond seeds, while promoting vigorous vegetative growth of the resulting seedlings.

CONCLUSION

The simultaneous application of scarification, moist chilling, and gibberellic acid is highly effective in increasing both the percentage and speed of seed germination in wild almond seedlings, as well as enhancing their vegetative growth. This integrated approach can be recommended for nurseries and conservation programs aimed at propagating wild almond species, primarily because it promotes better seedling establishment and growth under diverse environmental conditions. Further research should focus on determining the optimal concentrations and timing of each treatment to maximize efficiency across different seed batches.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

DATA AVAILABILITY

All data analyzed and generated during this study are included in this published article.

ETHICAL STATEMENT

Not applicable.

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