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Germination Performance of Wild Lupine Seeds (*Lupinus varius* L.) in Jordan Using Different Breaking Dormancy Treatments

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ABSTRACT

Wild lupine (*Lupinus varius* L.) is an annual plant native to the Mediterranean region. Only a few studies have investigated the germination performance of wild lupine seeds in the Mediterranean region and examined different effects of temperatures and multiple dormancy-breaking treatments, which could help crop breeders to develop varieties more resilient to climate change. This study was conducted to assess how different dormancy-breaking treatments, including hot water (80 °C), chemical scarification using sulfuric acid (H_2SO_4), and mechanical scarification, affect the germination rate and radicle length of wild lupine seeds when exposed to four controlled temperatures: 10, 14, 18, and 22 °C. A complete randomized design with three replications was used in this study. Mechanical scarification combined with a temperature of 22°C for 15 days was the most effective treatment for breaking the dormancy and resulted in the highest germination percentage (93.3%) and the highest radicle length (7.6 mm) for lupine seeds. In contrast, the lowest germination percentage (13.1-13.3%) was observed for H_2SO_4 and control wild lupine seeds (untreated) incubated at 10°C. In conclusion, mechanical scarification is an excellent method for breaking the seed dormancy of wild lupine seeds.

Keywords: Chemical scarification, germination rate, mechanical scarification, seed dormancy, radicle length.

INTRODUCTION

The Mediterranean climate of Jordan is predominantly semi-arid, with significant variations in temperature and precipitation across regions (Navarro et al., 2014; Al-Ghzawi et al., 2018; 2019a & b). Understanding how different temperature regimes influence the germination of wild lupine (*Lupinus varius*) seeds is essential in this context. Recently, *Lupinus varius* was assessed as a threatened species (Planchuelo, 2020). *Lupinus*, commonly known as lupine, comprises over 267 species, including annuals, perennials, evergreen shrubs, and subshrubs. These species encompass both wild and cultivated varieties (Mahfouze et al., 2015; Sholars & Riggins, 2022).

These plants are primarily found in the Mediterranean region, North Africa, and America (Drummond et al.,

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2012; Ačko and Flajšman, 2023). They thrive in arid hilly grasslands, coastal sands, cliffs, and alongside streams and rivers. In the Levant region (Lebanon, Palestine, Jordan, and Syria), the salty and chilled lupine beans are known as Turmus and are enjoyed as a healthy and delicious snack (Al-Eisawi, 1982; Kenchet et al., 2020; Knight et al., 2012). Additionally, lupines are widely cultivated as ornamental plants around the world due to their attractive flower spikes. Pantsyreva (2019) included *Lupinus* in a list of potentially valuable plants that could enhance domestic plant assortments and be used for landscaping. With its stunning blooms arranged on a long raceme, *Lupinus* shows great promise as an ornamental bedding plant and a specialty cut flower.

Lupines are members of the flowering legume family Fabaceae (synonym: *Leguminosae*) (Al-Eisawi, 1982) and are known for their ability to fix atmospheric nitrogen through symbiotic relationships with nitrogen-fixing bacteria. This unique trait not only enriches soil fertility but also enhances their role in ecological restoration (Marrs et al., 1982; Halvorson et al., 2005; Ahmed et al., 2023), particularly in regions with nutrient-poor soils like many areas of Jordan. Three *Lupinus* species have been identified and are naturally grown in Jordan; namely, *Lupinus angustifolius* L., *Lupinus luteus* L., and *Lupinus varius* L. (Al-Eisawi, 1982). *Lupinus palaestinus* is the most widely distributed species (Taifour, 2017). The latter taxon of ssp. *orientalis* (Franco et Silva) is an annual herb, growing in the Mediterranean, North Africa, South Europe, West Syria, and the Palestine region (Wink et al., 1995). *L. varius*, a botanical synonym of *L. angustifolius*, is a short to medium soft hairy annual that has the potential for use as a new cut flower crop, possessing inflorescences on the main stem and branches with blue flowers that are longer than those of some other species of Lupinus (Ogtr, 2021).

Wild lupine is one of several species threatened by the unregulated harvesting in Jordan (Al-Eisawi, 1982). To conserve and restore this species effectively, it is essential to understand its germination requirements (Swiecicki et al., 2000; Karaguzel et al., 2004). By gaining insight into the factors that influence wild lupine seed germination, we can refine conservation strategies, improve ecosystem health, and enhance biodiversity in Jordan's unique and delicate environments (Swiecicki et al., 2000; Ahmed et al., 2023).

The germination performance of wild lupine seeds, influenced by temperature conditions and dormancy-breaking techniques, significantly impacts their adaptation, distribution, and contribution to ecosystem functioning (Geneve, 2003; Karaguzel et al., 2004; Bermúdez-Torres et al., 2015; Jaganathan, 2022). Additionally, these wild seeds, belonging to the smooth-seeded Mediterranean and North African group (Cowling et al., 1998), often exhibit induced dormancy, a well-known physiological state that prevents immediate germination even under suitable conditions (Ogtr, 2021; Jaganathan, 2022). The presence of dormancy mechanisms underscores the complexity of lupine germination behavior and calls for a deeper investigation into effective dormancy-breaking strategies (Jaganathan, 2022).

Therefore, this study aimed to evaluate the germination performance of Jordanian wild lupine (*Lupinus varius* L.) seeds under various temperature regimes and dormancy-breaking treatments. By investigating the specific temperature ranges and techniques that promote lupine seed germination and seedling establishment in semi-arid climates.

Materials and Methods

Wild lupine seeds:

Seeds of wild lupine (*Lupinus varius* L.) used in this study were obtained from the Seed Bank at the National Agricultural Research Center (NARC), Al Baq'a, Jordan. This wild type was chosen because several other wild lupine types have previously been observed to respond to dormancy-breaking treatment (Jaganathan, 2022). This experiment was conducted in the NARC laboratory using a control climate chamber (Memmert HPP110, Germany) under different temperature regimes of 10, 14, 18, and

22°C for 15 days, with a 24 h photoperiod and relative humidity of approximately 30/40% (day/night).

Growth conditions and statistical analysis:

The experiment was designed with a complete randomized design with three replications. The lupine seeds were exposed to five treatments to break seed dormancy: 1) Hot water (HW), where seeds were immersed in 80°C hot water for 5 minutes and then cooled; 2) Chemical scarification (CS) using concentrated H_2SO_4 (96%) for 5 minutes; 3) Mechanical scarification (MS), where seeds were entirely scratched with 220-grit sandpaper for one minute to break the seed coat; 4) Stratification (ST), where seeds were moistened and stored at 4°C; and 5) Control treatment (CO), where no treatment was applied to seeds. The treated seeds in each treatment were incubated under different temperature regimes: 10, 14, 18, and 22°C for 15 days each.

For each replicate of every treatment, five Lupinus seeds were placed on filter paper within 9 cm diameter Petri dishes. Each treatment was repeated three times. After 15 days after planting (DAP), the percentage of seed germination and radicle length were measured.

Statistical analysis was conducted using the PROC GLIMMIX procedure of SAS 9.4 to characterize the factors that contribute to the percentage of seed germination and radicle length. The replications were considered random effects. The seed treatments (HW, CS, MS, ST, and CO) and the temperature regimes (10, 14, 18, and 22 °C) were considered as fixed effects. First, each trait was fitted to the following model:

$$y_{ijk} = \mu + T_i + C_j + R_k + TC_{ij} + \varepsilon_{k(ij)}$$

Where: y_{ijk} denotes the value of the trait of the ith treatment, the jth temperature, in the kth replication. The term μ is the grand mean, T_i is the treatment effect, C_j is the temperature effect, R_k is the effect of the kth replication, TC_{ij} terms are the interaction between treatments and temperatures, and $\varepsilon_{k(ij)}$ is the residual.

Comparison of means was conducted using the Tukey-Kramer method at a statistical significance threshold of $P<0.05$ to determine the differences in the performance of each treatment and its combinations.

Results:

The results showed the effects of different methods used to break the dormancy of wild lupine seeds on their germination and radicle length. MS showed significant variation compared to all other treatments (Table 1). The highest germination percentage was observed when MS (80%) was used, followed by HW (58%) and CS (55%). No significant difference was found between HW and CS treatments. Both HW and CS treatments had significantly higher germination percentages than seed stratification (ST), which had the lowest germination percentage, similar to the control (CO), where seeds were untreated (Table 1).

The MS significantly increased the radicle length (4.1 mm) compared with other treatments (Table 1). The results of this study indicated that seed germination and radicle length increased with increasing temperature (Table 2), suggesting that wild lupine seeds had better growth performance under 22°C. The highest temperature (22°C) had a significant effect on seed germination ($P < 0.05$). The highest germination occurred at 22°C, followed by 18, 14, and 10°C, respectively.

There were significant effects of dormancy-breaking treatments, temperatures, and treatments × temperature interaction on the seed germination percentage and radicle length of lupine seeds (Tables 1 and 3). The combination of MS and incubation at 22°C resulted in a significant increase in radicle length (7.6 mm) and the highest percentage of seed germination (93.3%). This was followed by HW and CS treatment for seeds incubated at 22°C. The combination of the CO and incubation at 10°C resulted in the lowest percentage of seed germination (13.3%) and the shortest radicle length (0.046 mm) (Table 3).

Figure 1 shows a high correlation for germination percentage of wild lupine seeds (*Lupinus varius* L.) under different dormancy-breaking treatments and illustrates clear relationships between temperature and germination.

The HW treatment has a linear regression model with an r^2 of 0.99, indicating a constant increase in germination percentage with increasing temperature (Figure 1a). However, the nonlinear trend is typical for untreated seeds (CO), whose temperature response has a natural limit (Figure 1b). The CS treatment also follows a linear regression model that shows a strong positive correlation between temperature and germination rate, with an r^2 of 0.96 (Figure 1a). ST shows the same trend, which implies a consistent increase in germination rate with temperature, although the rate of increase may slow at higher temperatures (Figure 1a). This model fits the data almost perfectly and suggests a consistent response across the entire temperature range. Finally, MS follows a quadratic model showing that germination rates increase with temperature up to a certain point; after a certain point, the increase slows down (Figure 1b). However, this treatment results in the highest germination rates overall, demonstrating its effectiveness in breaking dormancy. Overall, the HW, ST, and CS treatments exhibit strong linear relationships, where germination percentages rose steadily with temperature. In contrast, the CO and MS treatments follow quadratic trends, characterized by an initial rise followed by a plateau or slight decline at higher temperatures. Among all treatments, MS, in conjunction with higher temperatures, results in the highest germination percentage, making it the most effective dormancy-breaking treatment for wild lupine seeds.

Discussion:

Seed dormancy is an innate seed property and is incompletely understood. The results of this study have significant implications for enhancing the germination and growth of wild lupine seeds. Our findings indicated that the most effective treatment for breaking dormancy was MS, particularly when combined with incubation at 22°C, where the germination percentage was 93.3% (Table 3). The second highest germination percentage 86% was obtained with CS under 22°C. Hilooğlu et al. (2018) and Paim et al. (2021) reported that exposing plants to MS can be used as an effective method to break dormancy. Similarly, in many plant species, the MS method was the best method to overcome seed coat impermeability (Safiri et al., 2012; Asaadi et al., 2015). Further investigation into the optimal duration and intensity of CS would also be beneficial (Table 3).

Lupine seeds commonly exhibit both physical and physiological dormancy. Various methods of scarification, such as mechanical scratching and acid treatment, have been investigated to overcome physical dormancy (Hilooğlu et al., 2018; Jones et al., 2010; Karaguzel et al., 2004; Paim et al., 2021). Similarly, hormonal treatments using gibberellic acid and stratification have been employed to break physiological dormancy (Karaguzel et al., 2004).

On the other hand, this present study highlights the role of temperature in germination and root development. The HW treatment has a linear regression model with an r^2 of 0.99 (Figure 1a). This strong adaptation suggests a predictable and consistent response to temperature in this treatment. In contrast, CO treatment (Figure 1b) follows a quadratic model, indicating a more complex relationship where the germination rate increases with temperature up to a certain point before decreasing. The CS treatment shows a strong positive correlation between temperature and germination percentage, with an r^2 of 0.96 (Figure 1a). This treatment promotes germination as the temperature increases, reflecting its effectiveness in promoting seed response to temperature changes. Warmer temperatures lead to higher germination rates and improved radicle formation. Previous research has extensively examined the effect of temperature on seed germination for wild *Lupinus* seeds of various genera (Geneve, 2003). This emphasizes the importance of temperature regulation in creating optimal conditions for lupine seed germination. In Jordan and the entire Mediterranean region, significant seasonal variations in temperature play a critical role in determining the ideal timing for seedling establishment (Hatzilazarou et al., 2023). By simulating the conditions of our laboratory incubator, it was possible to estimate the ideal germination temperatures found in nature. These findings have potential applications in reforestation and conservation projects for wild lupine. However, it is essential to consider regional environmental conditions and potential interactions with other plant species to fully understand and utilize these findings.

In summary, the germination performance of Jordanian wild lupine (*L. varius*) seeds is influenced by temperature conditions (Geneve, 2003) and dormancy-breaking mechanisms (Jaganathan, 2022). Understanding these factors is crucial for conservation and restoration initiatives (Marrs et al., 1982; Swiecicki et al., 2000).

Ecological Implications:

Understanding the dynamics of lupine germination is crucial for managing lupine populations and restoring ecosystems. The ability of lupine seeds to germinate under different temperature conditions and break seed dormancy has implications for their adaptation to changing climates and successful colonization of various habitats (Bermúdez-Torres et al., 2015; Jaganathan, 2022; Paim et al., 2021). The successful restoration of degraded landscapes depends on the effective germination and establishment of native plant species (Greipsson and El-Mayas, 2003). Our results indicated that germination patterns and dormancy-breaking mechanisms in wild lupine can provide valuable information for restoration practices and improve the success of re-vegetation efforts.

Conclusions:

The study showed that mechanical scarification was the most effective method for breaking the dormancy of lupine seeds and promoting their germination. Water and chemical scarification treatments were also found to be positively associated with seed germination percentage and radicle length, although they were slightly less successful than mechanical scarification. Using hot water can potentially weaken seed coats and break down inhibitors that impede germination. The combination of mechanical scarification and germination at 22 °C produced the highest seed germination percentage and the greatest radicle length.

Authors' Contributions:

WO and KA performed the data analyses and wrote the manuscript. Ahmad Al-edwan conducted the experiments. Abdul Al-Ghazawi, MA, and AJ conceived of the study. KA supervises the experiment's operation. AR edited the manuscript. All authors read and approved the final manuscript.

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Disclosure Statement:

The authors disclose no conflict of interest.

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APPENDICES

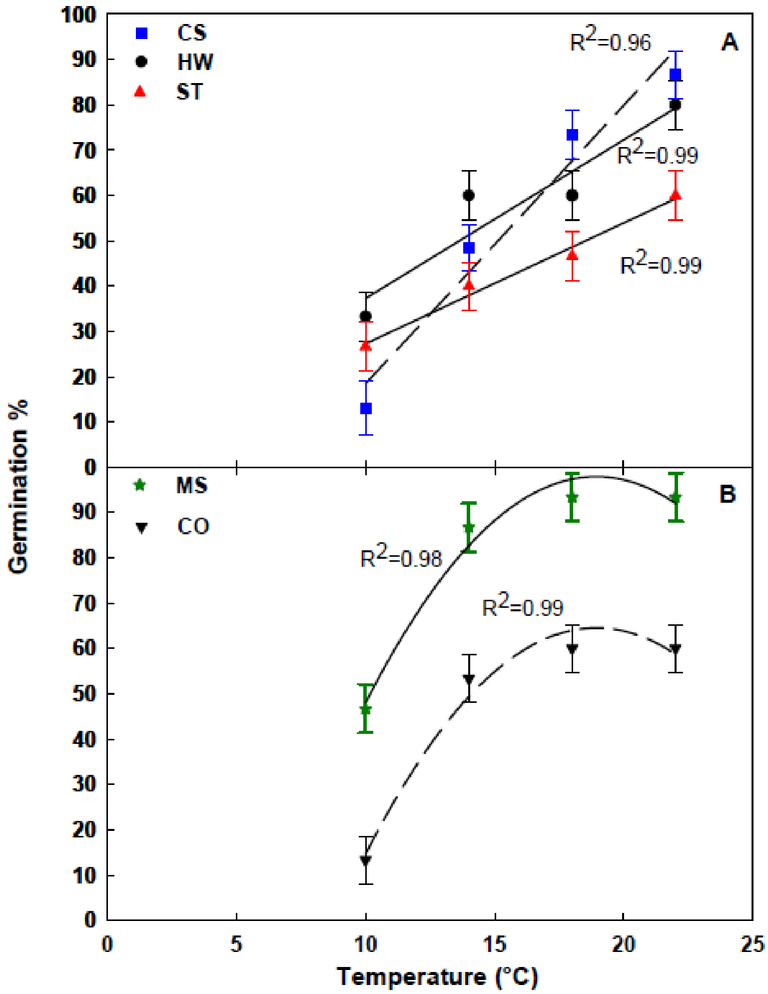

Figure 1. Correlation coefficient between temperature (°C) and germination % for different dormancy-breaking treatments applied to wild lupine seeds. (A) Chemical scarification using concentrated H_2SO_4 (CS, Y= 6.1408x- 42.867), hot water (HW, Y= 3.6353x, stratification (ST, Y=2E-15x^2+2.6667x+0.6667); (B) mechanical scarification (MS, Y= -0.625x^2+23.667x-126.17), control (CO, Y=-0.625x^2+ 23.667x-159.5).

Table 1. Effect of different breaking-dormancy treatments on germination of wild lupine seeds (*Lupinus varius* L.) and radicle length.

Treatments	Seed germination %	Radicle length (mm)
HW	58.3 b	3.2 ab
CS	55.4 b	2.6 b
MS	80.0 a	4.1 a
ST	43.3 c	2.3b
CO	46.7 c	2.4 b

Comparison of means was conducted using the Tukey-Kramer method at a statistical significance threshold (P<0.05).
Variables with a different letter, the difference is statistically significant at (P<0.05 significance level.
Hot water (HW), chemical scarification using conc. H_2SO_4 (CS), mechanical scarification (MS), stratification (ST), in addition to the control (CO) in which seeds remained untreated.

Table 2. Effect of different temperatures on germination of wild lupine seeds (*Lupinus varius* L.) and radicle length.

Temperature (°C)	Seed germination (%)	Radicle length (mm)
10	26.6d	0.3 d
14	57.7c	1.9 c
18	66.7 b	3.2 b
22	76.0 a	6.3 a

Comparison of means was conducted using the Tukey-Kramer method at a statistical significance threshold (P<0.05).
Variables with a different letter, the difference is statistically significant at (P<0.05) significance level.

Table 3. Effect of different combinations of breaking the dormancy treatments and temperatures on germination of wild lupine seeds (*Lupinus varius* L.) and radicle length.

Parameter / Treatment	Seed germination (%)				Radicle length (mm)			
	Temperature (°C)							
	10	**14**	**18**	**22**	**10**	**14**	**18**	**22**
HW	33.3 fgh	60.0 cd	60.0 cd	80.0 ab	0.3 e	2.4 de	3.4 bcde	6.6 ab
CS	13.1 hi	48.5 def	73.3 bc	86.7 ab	0.5 e	1.0 e	2.7 cde	6.2 abc
MS	46.7 def	86.7 ab	93.3 a	93.3 a	0.3 e	3.3 bcde	5.1 abcd	7.6 a
ST	26.7 ghi	40.0 efg	46. 7 def	60.0 cd	0.3 e	1.4 e	2.4de	5.3 abcd
CO	13.3 i	53.3 de	60.0 cd	60.0 cd	0.04 e	1.5 e	2.5 de	5.7 abcd

Comparison of means was conducted using the Tukey-Kramer method at a statistical significance threshold (P<0.05).
The interaction of different treatments and at different temperatures. Hot water (HW), chemical scarification using conc. H_2SO_4 (CS), mechanical scarification (MS), and stratification (ST), in addition to the control (CO), in which seeds remained untreated.

إنبات بذور الترمس البري (*Lupinus varius* L.) في الأردن تحت طرق كسر سكون مختلفة

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ملخص

يعتبر الترمس البري (*Lupinus varius L.*) نباتا حوليا ينتشر في منطقة حوض البحر الأبيض المتوسط والتي تعتبر موطنه الأصلي. قليلة هي الدراسات التي إهتمت بدراسة إنبات بذور الترمس البري في منطقته من حيث كسر طور سكون البذور خصوصا تلك المتعلقة بإستخدام درجات الحرارة المختلفة. تعتبر عملية كسر طور السكون مهمة جدا حيث أنها تمكن مربي المحاصيل من إنتاج وتطوير أصناف أكثر مرونة في مواجهة التغيرات المناخية المختلفة. تهدف هذه الدراسة إلى تقييم تأثير طريقة كسر طور السكون في نبات الترمس البري بإستخدام معاملات مختلفة تشمل الماء الساخن (80 م°)، التخديش الكيميائي (H_2SO_4)،التخدش الميكانيكي، والتنضيد (5 م°) على إنبات البادرات والجذير لبذور الترمس البري تحت أربع درجات حرارة مختلفة (10، 14، 18 و22 م°) لمدة 15 يوم لكل درجة حرارة في الحاضنة.

في هذه الدراسة أستخدم التصميم العشوائي الكامل بثلاثة مكررات. أظهرت نتائج هذه التجربة أن التخديش الميكانيكي على درجة حرارة 22 م° لمدة 15 يومًا كان أفضل طريقة في عملية كسر طور السكون وكان ذلك واضحا من خلال زيادة إنبات البادرات (93.3%) و زيادة طول الجذر الوتدي (7.6 ملم) لنبات الترمس. بالمقابل في معاملة التخديش الكيميائي تم تسجيل أدنى إنبات للبادرات (13.1 – 13.3%) عند درجة حرارة 10م°.أوضحت هذه الدراسة أن التخديش الميكانيكي هو الطريقة المثالية لكسر طور السكون في بذور الترمس البري.

الكلمات الدالة: التخديش الكيميائي، نسبة الإنبات، التخديش الميكانيكي، طور السكون، طول الجذير الوتدي.

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