



# RESEARCH COMMUNICATION

# Overcoming physical dormancy: Optimizing seed germination in *Helianthemum* and *Cistus* species for desert truffle mycorrhizal seedling production

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#### **Abstract**

Seed germination is a critical stage in the plant reproductive cycle, significantly influencing species fitness. Variations in germination rates are often considered adaptations to specific ecological conditions. Understanding this process is essential for developing effective conservation strategies, particularly for producing mycorrhizal seedlings in greenhouse settings. A key challenge in desert truffle cultivation is the low germination rate of seeds from their host plants, which impedes the establishment of mycorrhizal plants under greenhouse conditions. This study investigates the germination behaviour of four *Helianthemum* species (*H. lippii*, *H. salicifolium*, *H. ledifolium*, *H. almeriense*) and two *Cistus* species (*C. ladanifer*, *C. laurifolius*) under controlled conditions. Three pre-sowing treatments, manual scarification, soaking intact seeds in distilled water for 24 hr at ambient temperature and sulphuric acid treatment were applied. Among all *Helianthemum* and *Cistus* species tested, manual scarification yielded the highest germination percentages. While other pre-sowing treatments promoted germination in some species, none matched the efficacy of manual scarification. The consistently high germination rates following mechanical disruption of the seed coat suggest that dormancy is primarily governed by seed coat impermeability. Physical dormancy, resulting from an impermeable seed coat, appears to be the primary cause of low germination in untreated seeds of the studied *Helianthemum* and *Cistus* species.

**Keywords:** conservation strategies; desert truffles; ecological adaptation; germination rates; greenhouse cultivation; pre-sowing treatments; seed coat impermeability

# Introduction

Seed germination is a vital event within the life cycle of a plant, which is crucial for the fitness of the species. Variations in germination rates have been interpreted as adaptations to specific ecological conditions (1). Seed coat-imposed dormancy, characterized by hardness and impermeability to water, is a key mechanism behind primary dormancy in several species of the genera Helianthemum and Cistus (2, 3). Specifically, the physical dormancy in Cistaceae species is associated with the presence of a hard, lignified seed coat, which prevents water uptake a trait particularly pronounced in Mediterranean climates (3). Dormancy is defined as the inability of a seed to germinate, even under conditions typically considered favourable for germination. Seed dormancy caused by a water-impermeable seed coat is termed physical dormancy, which develops during seed maturation and drying or during fruit development (4-6). Physical dormancy is a widespread mechanism in species adapted to arid environments, ensuring germination occurs only under optimal moisture conditions (4). Understanding the germination process is crucial for developing conservation strategies, particularly those aimed at producing mycorrhizal seedlings in greenhouse settings. This is especially relevant given that plant colonization is a process that depends on both the availability of seeds and local conditions for

seedling establishment (7-11). For instance, the seed dormancy mechanisms such as those in Cistaceae enhance species persistence in unpredictable environments by spreading germination over time (9).

Numerous studies indicate that mycorrhizas enhance the drought tolerance of plants. Most ectomycorrhizal plants are trees and woody shrubs; however, a few herbaceous and shrubby plants are known to form ectomycorrhizas, including certain members of the Cistaceae, such as H. salicifolium (L.) Mill., H. ledifolium (L.) Mill. and H. lippii (10, 12, 13). Helianthemum species form ectomycorrhizal associations with desert truffle fungi that significantly improve phosphorus acquisition in nutrient-poor soils (12). Similarly, Cistus species, notably C. ladanifer and C. laurifolius form ectomycorrhizal associations with fungi such as Tuber and Terfezia, which are critical for their adaptation to Mediterranean environments characterized by drought and nutrient-poor soils (14, 15). Terfezia associations with Cistus species enhance seedling survival under water stress by improving hydraulic conductivity and root morphology (14). These mycorrhizal plants play a critical ecological role in the maintenance of Mediterranean shrublands and xerophytic grasslands by mitigating erosion and desertification (16). Among the most commonly reported mycorrhizal associates of SHEIBANI & JAMALI 2

Helianthemum spp. are the hypogeous fungi Tirmania, Terfezia and Picoa (17). These fungi form extensive mycorrhizal networks with Helianthemum, facilitating water and nutrient transfer in arid ecosystems (17). For Cistus spp., ectomycorrhizal fungi enhance nutrient uptake and water-use efficiency, contributing to their resilience in arid ecosystems (14, 18). Also, these species exhibit a high degree of mycorrhizal specificity, with certain fungal partners optimizing their performance in calcareous soils (18). The symbiotic relationship between plant roots and these fungi is undoubtedly one of the most widespread associations in terrestrial ecosystems. Ectomycorrhizal Helianthemum and Cistus seedlings often exhibit greater growth compared to non-mycorrhizal plants under cyclic drought conditions. Furthermore, studies consistently demonstrate higher survival rates or faster recovery from drought stress in ectomycorrhizal seedlings compared to non-mycorrhizal plants (19). Seed coat hardness and impermeability to water are likely the primary causes of dormancy in several Cistaceae species (3). Treatments such as heat and scarification can release these seeds from dormancy by disrupting the impermeable seed coat, thereby allowing imbibition and germination to proceed (3, 20). Scarification effectively breaks physical dormancy in Cistus species, enabling rapid germination under controlled conditions (20). Despite their ecological importance, relatively few studies have explored the reproductive biology of Helianthemum and Cistus species (3, 21-23). Limited understanding of seed bank dynamics in Cistaceae, which further complicates conservation efforts in Mediterranean ecosystems (21).

This study aims to investigate the seed germination behaviour of four *Helianthemum* species (*H. salicifolium*, *H. ledifolium*, *H. lippii* and *H. almeriense*) and two *Cistus* species (*C. ladanifer* and *C. laurifolius*) under controlled conditions, with a focus on evaluating the efficacy of various pre-sowing treatments to overcome physical dormancy. Additionally, it seeks to elucidate the role of seed coat impermeability in limiting germination and to provide insights into optimizing mycorrhizal seedling production for ecological restoration and desert truffle cultivation in greenhouse settings. Specifically, this study examines the potential of enhanced germination to support the establishment of ectomycorrhizal associations in both *Helianthemum* and *Cistus* species, thereby facilitating the production of robust host plants for desert truffle cultivation (14, 18).

## **Materials and Methods**

## Seed collection

Ripe fruits (capsules) containing mature seeds of *H. lippii*, *H. ledifolium* var. *ledifolium* and *H. salicifolium* var. *salicifolium* were manually collected during March 2020 from a natural rangeland population in the Lamerd region, Fars Province, Iran (27°46′N, 53° 57′E; altitude: 944 m). Seeds of three additional species, *H. almeriense*, *Cistus laurifolius* and *C. ladanifer* were obtained in 2020 from a Spanish seed supplier, SEMILLAS CANTUESO. These *Helianthemum* and *Cistus* species were selected as they are adapted to Mediterranean and semi-arid environments, host desert truffles and have economic value. Seeds were cleaned by sieving to remove debris and inspected for deformities and viability using a binocular microscope. Seed viability was initially assessed using the moist filter paper method to evaluate germination potential (4).

Dormancy was inferred through microscopic examination of seeds with normal morphology, which helped distinguish dormant seeds from non-viable ones (4). Seeds were stored in paper envelopes under dry conditions (relative humidity < 20 %) at 20 °C- 25 °C until analysis.

## **Pre-sowing seed treatments**

Seeds were subjected to various mechanical, physical and chemical pre-sowing treatments. Mechanical scarification was performed by rubbing the seeds between two sheets of fine-grained sandpaper (grit size 240) for 30 sec with consistent manual pressure, ensuring uniformity across all seeds to facilitate reproducibility, though exact force was not quantified due to the absence of a force gauge (23). Physical scarification involved soaking intact seeds in distilled water for 24 hr at ambient temperature (20 °C- 25 °C). Chemical scarification was conducted by soaking intact seeds in concentrated sulphuric acid (98 % H<sub>2</sub>SO<sub>4</sub>) for 30 sec, 2, 3 or 5 min. Seeds treated with H<sub>2</sub>SO<sub>4</sub> were thoroughly rinsed three times in sterile distilled water before culture. Safety measures included conducting procedures in a well-ventilated fume hood, with authors wearing protective gloves and goggles; acid was handled in pre-measured aliquots, spills neutralized with sodium bicarbonate and direct handling or dilution avoided, with seeds submerged under controlled conditions. Post-treatment, seeds exposed to H<sub>2</sub>SO<sub>4</sub> were rinsed three times with sterile distilled water. Germination tests were conducted in 9 cm diameter disposable Petri dishes on moist Whatman No. 41 filter paper, at a temperature of 22 °C - 25 °C. For each treatment, three replicates were used, with untreated seeds serving as controls. Germination, defined by radicle emergence (24), was recorded daily over a 20-day period. The average time to germination, which ranged from 6 to 12 days, was calculated to inform subsequent greenhouse experiments and synchronize seedling development.

#### **Statistical analysis**

Data were statistically analysed using Statistical Package for the Social Sciences (SPSS) version 17.0, calculating mean germination percentage, standard error and average post-treatment germination times (6 - 12 days) to support greenhouse and synchronization needs

The mean germination percentage, its standard error and the average germination time post-treatment were calculated to provide comprehensive insights for greenhouse application and seedling synchronization. One-way analysis of variance (ANOVA) assessed treatment effects at p < 0.05, with Tukey's HSD test identifying significant differences. Normality (Shapiro-Wilk) and variance homogeneity (Levene's test) were confirmed prior to ANOVA.

## **Results**

The final germination percentage of seeds germinated without pretreatment (control) varied considerably across species, ranging from 6.66 % (*H. lippii*) to 10.33 % (*H. salicifolium*) (Table 1). Statistical analysis revealed a significant difference among the treatments applied for seed germination across all studied plant species. The highest germination percentages of pretreated seeds were observed with manual scarification (sandpaper), yielding 66.66 % (*H. lippii*), 66 % (*H. almeriense*), 70.66 % (*C. ladanifer*), 66 % (*C. laurifolius*), 95.66 % (*H. salicifolium*) and 95 % (*H. ledifolium*), respectively (Fig. 1). In *H.* 

**Table 1.** Comparison of the mean of each treatment between plant species

Plants	Control	Sandpaper	H <sub>2</sub> SO <sub>4</sub> (30 sec)	H <sub>2</sub> SO <sub>4</sub> (2 min)	H <sub>2</sub> SO <sub>4</sub> (3 min)	H <sub>2</sub> SO <sub>4</sub> (5 min)	Water soaked (24 hr)
H. lippii	$6.66 \pm 1.28^{a}$	$66.66 \pm 2.48^{a}$	$30.66 \pm 1.87^{a}$	$39.33 \pm 2.03^{a}$	$24.66 \pm 1.74^{a}$	$31.66 \pm 2.04^{a}$	$35.00 \pm 0.94^{a}$
H. almeriense	$8.00 \pm 1.28^{a}$	$66.00 \pm 2.48^a$	$37.66 \pm 1.87^{a}$	$36.33 \pm 2.03^{a}$	28.66 ± 1.74 <sup>a</sup>	$34.66 \pm 2.04^{a}$	$18.33 \pm 0.94^{b}$
C. ladanifer	$7.66 \pm 1.28^{a}$	$70.66 \pm 2.48^a$	$30.33 \pm 1.87^{a}$	$39.66 \pm 2.03^{a}$	27.33 ± 1.74 <sup>a</sup>	$30.66 \pm 2.04^{a}$	$17.00 \pm 0.94^{b}$
C. laurifolius	8.33 ± 1.28 <sup>a</sup>	$66.00 \pm 2.48^a$	$31.66 \pm 1.87^{a}$	$36.00 \pm 2.03^{a}$	27.33 ± 1.74 <sup>a</sup>	$33.33 \pm 2.04^{a}$	$17.33 \pm 0.94^{b}$
H. salicifolium	$10.33 \pm 1.28^{a}$	95.66 ± 2.48 <sup>b</sup>	$12.66 \pm 1.87^{b}$	$34.33 \pm 2.03^a$	$30.33 \pm 1.74^{a}$	$53.00 \pm 2.04^{b}$	$15.00 \pm 0.94^{b}$
H. ledifolium	$10.33 \pm 1.28^{a}$	95.00 ± 2.48 <sup>b</sup>	$10.66 \pm 1.87^{b}$	34.66 ± 2.03 <sup>a</sup>	29.33 ± 1.74 <sup>a</sup>	49.00 ± 2.04 <sup>b</sup>	15.66 ± 0.94 <sup>b</sup>

The lowercase letters are the difference between the treatments in each row for each plant.

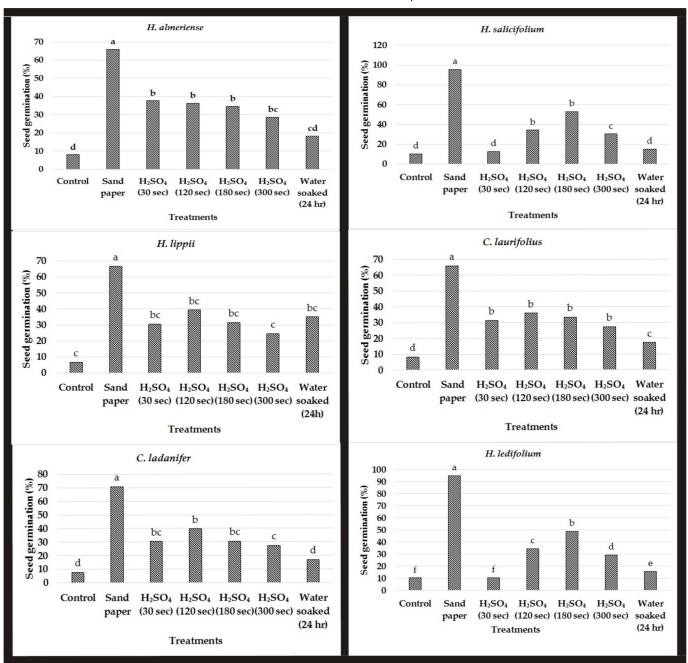


Fig. 1. Comparison of seed germination percentages across different species under various pre-sowing treatments.

*lippii*, water-soaking for 24 hr had a significantly positive effect on germination compared to the control. Both mechanical scarification (sandpaper) and water-soaking resulted in germination rates higher than those of unsoaked seeds for all species. The thinner seed coat of *H. lippii*, compared to *H. ledifolium* and *H. salicifolium*, likely contributed to the effectiveness of a shorter soaking duration for this species. Prolonged exposure to  $H_2SO_4$  may have caused embryo damage, potentially burning the seeds. There was no significant

difference in germination percentages among species following  $H_2SO_4$  treatments for 2 min and 3 min. However, at the 5 min  $H_2SO_4$  treatment, H. salicifolium and H. ledifolium showed significantly higher germination rates (approximately 40 % and 30 % respectively) compared to other species, which ranged from 10 % to 20%.

Based on the observation that germination began on the first day (24 hr post-treatment) and continued until the  $12^{\text{th}}$  day

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across all treatments, with no germination observed by the  $20^{th}$  day, the average germination times post-treatment ranged from 6 to 12 days.

#### **Discussion**

Species within the Cistaceae family, including *Helianthemum* and *Cistus*, establish ectomycorrhizal, ectendomycorrhizal and endomycorrhizal associations with desert truffle fungi such as *Terfezia*, *Tirmania* and *Picoa* (10, 25). These symbioses enhance plant resilience to drought and nutrient scarcity, playing a vital role in stabilizing Mediterranean shrublands and xerophytic grasslands against erosion and desertification (14). The growing economic and ecological significance of desert truffles has spurred interest in their cultivation, necessitating the production of mycorrhizal seedlings in controlled greenhouse environments for subsequent field transplantation (26). A major bottleneck in this process is the low germination rate of host plant seeds, predominantly due to physical dormancy induced by an impermeable seed coat.

This study confirms that physical dormancy, driven by seed coat impermeability, is a predominant trait in the investigated *Helianthemum* and *Cistus* species. Mechanical scarification proved to be the most effective pre-sowing treatment, achieving germination rates of 90 % - 100 % within 24 hr across all species. This near-complete germination highlights the seed coat as the principal barrier to water uptake, a characteristic widely documented in Cistaceae (4). In untreated seed lots, germination ranged from 6.66 % to 10.33 %, underscoring the adaptive significance of hardseededness in arid ecosystems. This mechanism likely delays germination until favourable moisture conditions prevail, thereby enhancing seedling establishment in unpredictable Mediterranean climates (9).

Alternative treatments yielded variable outcomes. Water soaking for 24 hr significantly enhanced germination in *H. lippii*, likely due to its thinner seed coat relative to *H. salicifolium* and *H. ledifolium*. Sulphuric acid treatments elicited species-specific responses, with *H. salicifolium* and *H. ledifolium* showing elevated germination rates (40 % and 30 % respectively) after 5 min, compared to 10 % - 20 % in other species. This variability may stem from differences in seed coat thickness, chemical composition or embryo tolerance, with prolonged acid exposure potentially causing embryo damage in less resilient species. These results corroborate prior research demonstrating the efficacy of scarification, hot water and acid treatments in breaking physical dormancy in Cistaceae (21-23).

The success of mechanical scarification has far-reaching implications for ecological restoration and the commercial production of mycorrhizal seedlings. By permeabilizing the seed coat, this method enables rapid, uniform germination, a prerequisite for scalable greenhouse production. In natural settings, seed coat impermeability is gradually overcome through environmental factors such as temperature fluctuations, wetting-drying cycles and soil abrasion (4), mirroring the effects of manual scarification. This suggests that physical dormancy serves as an ecological strategy to stagger germination, optimizing survival in harsh conditions.

The transition to field conditions in arid regions requires consideration. Soil heterogeneity, with varying texture, organic matter and nutrients may influence natural seed coat breakdown (27), with sandy soils delaying germination more than loamy soils

with pronounced wetting-drying cycles. Erratic rainfall, a feature of arid seasonal water availability may further delay unscarified seed germination (28). Scarification could ensure timely germination, enhancing mycorrhizal resilience under fluctuating moisture (4). Future field trials across diverse soils and climates are needed to refine practices like irrigation or amendments. In controlled settings, however, scarification ensures consistency and aids in synchronizing seedling emergence.

Optimizing germination in *Helianthemum* and *Cistus* is key for desert truffle cultivation and restoration, with scarification supporting robust ectomycorrhizal associations that improve seedling resilience to water and nutrient stress (14). This facilitates reintroduction into arid landscapes, aligning with sustainable goals. Future research should refine techniques to minimize embryo damage, investigate genetic factors in seed coat impermeability and evaluate long-term field performance.

#### **Conclusion**

This study establishes seed coat impermeability as the primary germination constraint in Helianthemum and Cistus species, with mechanical scarification proving highly effective in overcoming physical dormancy. Achieving near-complete germination addresses a critical hurdle in mycorrhizal seedling production, enhancing desert truffle cultivation, climate resilience and biodiversity conservation. The method's rapid, uniform germination accelerates greenhouse seedling production, supporting the scalable desert truffle industry and contributing to food security and economic diversification in arid regions. Resilient ectomycorrhizal associations bolster host plant adaptation to intensifying drought and heat stress, preserving Mediterranean shrubland stability against desertification. Conservation efforts benefit from reintroducing mycorrhizal seedlings to restore biodiversity and ecosystem services. These findings advance Mediterranean conservation and sustainable agriculture, necessitating further research into technique refinement and field performance under varied conditions.

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# **Authors' contributions**

MS conducted field experiments, sample preparation and laboratory works. SJ conceived the idea of the research, designed the scientific experiments and methodology, field surveys, data analysis using SPSS software, original draft preparation, its review and editing. All authors read and approved the final manuscript.

# **Compliance with ethical standards**

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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