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RESEARCH ARTICLE

Comparative analysis of seed dormancy breaking methods in *Sesuvium portulacastrum* (L.) L.

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Abstract

Seed dormancy is a significant challenge that hinders the successful germination of *Sesuvium portulacastrum*, a halophytic species with promising applications in phytoremediation and coastal stabilization. This study aims to evaluate the efficacy of several dormancy-breaking treatments on the germination of *S. portulacastrum* seeds. The treatments assessed include potassium nitrate, sulfuric acid, manual scratching, hot water treatment and gibberellic acid (GA_3) in promoting the germination of *S. portulacastrum* seeds. Each treatment was applied to seeds and germination rates were monitored to determine effectiveness. The results revealed that the treatment with concentrated sulfuric acid for 2 min significantly enhanced germination rates compared to the other methods tested. This method outperformed the others, highlighting its potential as an effective protocol for overcoming dormancy in *S. portulacastrum*. The findings of this research provide important insights into optimizing germination protocols for this species, which can contribute to its broader use in environmental applications, particularly in restoring degraded coastal areas and improving soil stability. By breaking seed dormancy effectively, *S. portulacastrum* can be utilized more effectively in restoration projects aimed at addressing environmental degradation and enhancing ecosystem resilience. Overall, this study underscores the importance of understanding and manipulating seed dormancy to promote the use of halophytic species in ecological restoration efforts.

Keywords

dormancy-breaking methods; germination enhancement; hard seed coat; seed dormancy; sulfuric acid treatment

Introduction

Sesuvium portulacastrum (L.) L., commonly known as sea purslane, is a versatile halophyte valued for its ecological and economic significance (1). This succulent perennial plant, native to coastal ecosystems, exhibits remarkable salt tolerance and adaptability to harsh environmental conditions, making it an essential species for research on plant adaptations to salt stress (2). Its ecological contributions include soil stabilization, salinity regulation and biodiversity enhancement, while its potential applications span phytoremediation, medicinal uses and nutritional supplementation due to its high antioxidant content (3). The plant's ability to thrive in saline soils (Fig. 1)

underscores its importance in addressing environmental challenges such as soil salinization and desertification.

Fig. 1. Morphology of *S. portulacastrum.*

Despite its ecological and economic importance, *S. portulacastrum* faces propagation challenges due to low seed germination rates, typically around 4% under natural conditions, primarily attributed to seed dormancy. Seed dormancy is an adaptive trait that regulates germination timing to ensure seedling survival under favorable environmental conditions (4). In *S. portulacastrum*, dormancy is often associated with physical barriers, such as a hard seed coat that restricts water uptake and gas exchange (5). This characteristic poses a significant limitation to seed-based propagation, which is particularly relevant for large-scale restoration projects and sustainable agricultural applications in saline-affected areas.

Efforts to overcome seed dormancy in *S. portulacastrum* have focused on physical, chemical and physiological methods, yet earlier studies lack comprehensive analysis on the optimization and scalability of these techniques. For instance, manual scarification has been shown to improve germination in hard-seeded legumes (6), but its labor-intensive nature limits its practicality for large-scale use. Similarly, chemical treatments like sulfuric acid scarification have demonstrated efficacy in breaking seed dormancy across species with hard seed coats, including *S. portulacastrum* (7). However, these studies often overlook the delicate balance between treatment concentration, exposure time and potential seed damage, leaving gaps in understanding the scalability and safety of these methods for widespread application.

Our study aims to address these shortcomings by systematically evaluating various dormancy-breaking treatments for *S. portulacastrum*, with a focus on identifying the most effective, practical and scalable methods. The results will advance the understanding of seed dormancy mechanisms and provide practical insights for enhancing germination success, thereby supporting conservation and restoration efforts. Additionally, this research contributes to the broader application of *S. portulacastrum* in agriculture and environmental management by overcoming propagation barriers.

Economic Importance of Sesuvium portulacastrum

Sesuvium portulacastrum is economically significant due to its multiple applications. In saline agriculture, it serves as a potential crop and cover plant, helping to manage soil salinity and improve agricultural productivity in salt-affected areas (8). The plant's antioxidant-rich leaves are being explored for their medicinal and nutritional properties, including their use in traditional medicine for treating wounds and as a source of bioactive compounds for health supplements (9). Moreover, its role in phytoremediation extends to heavy metal removal from contaminated soils, highlighting its potential in environmental cleanup and sustainable land management strategies (10). By integrating these diverse benefits, the species aligns with global efforts to promote sustainability in agriculture and environmental restoration (11).

Materials and Methods

Seed Collection and Storage

The collection of seeds is a critical step in any study related to seed germination and dormancy breaking, as the quality, viability and handling of seeds can significantly influence the outcomes of subsequent experiments. In (Fig. 2), Seeds of *Sesuvium portulacastrum* were collected from the Tamil Nadu Paper Limited, Karur and Compost

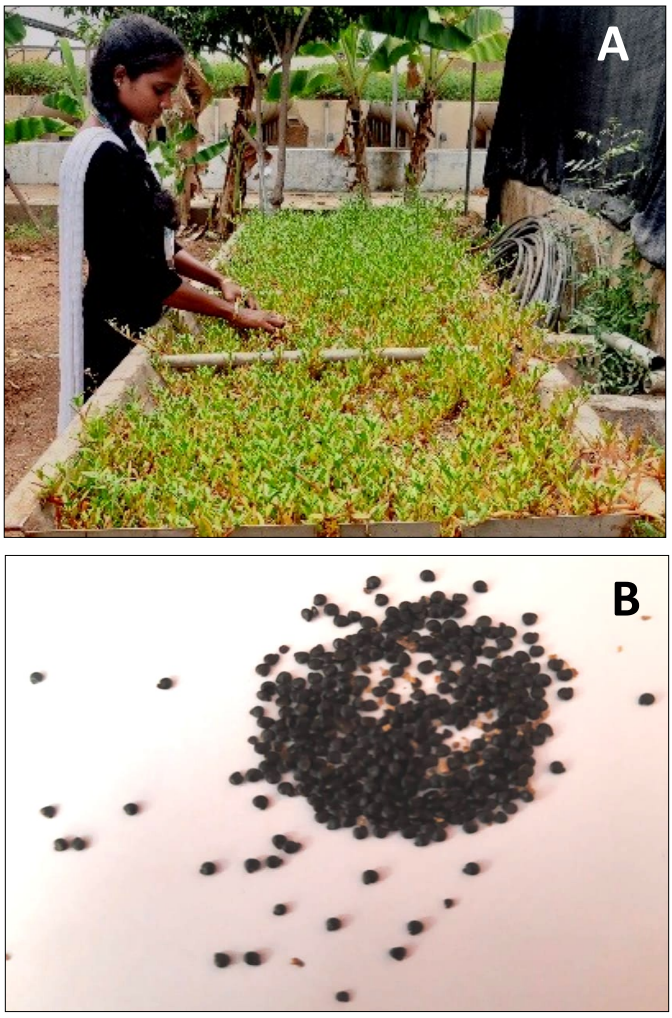

Fig. 2. *S. portulacastrum* seed collection. (**A**) Collection of seeds from mother bed, (**B**) Collected seeds.

yard, TNAU. Seed collection was conducted during the peak fruiting season to ensure maximum seed maturity, a factor known to influence germination success (12). Plants with ripening inflorescences were collected from the fields and air-dried at room temperature (27 ± 2) for 3 weeks and seeds were separated from the inflorescences by rubbing. After separation from the inflorescence, we confirmed that the seeds had high physical dormancy by conducting an initial germination test in distilled water. We also confirmed that these seeds were viable using tetrazolium chloride. The tetrazolium chloride test was performed using the protocol of (13). In this method, weed seeds were stored in 1% tetrazolium chloride solution for 48 hr in the dark at 30 °C. We observed the formation of red color around the embryo of most of the seeds tested indicating that a high percentage of the seeds were viable. The seeds used in this experiment were stored in a dry environment at a temperature of 25 °C for 2 months between harvest and conducting these experiments. During this time, after-ripening would have been occurring, releasing some but not all of the primary dormancy of these seeds, leading to the 27.33 ± 3.05% germination rate observed in the control.

Experimental design

The study was structured to systematically compare the efficacy of various dormancy-breaking treatments on the germination rate of *Sesuvium portulacastrum* seeds. Five dormancy-breaking treatments were evaluated. i) for Potassium Nitrate (KNO_3) treatment, seeds were soaked in 2% potassium nitrate solution for 10 min, 15 min and overnight to assess the impact of nitrate availability on seed dormancy, ii) for Sulfuric Acid (H_2SO_4) treatment, concentrated sulfuric acid was used to chemically scarify the seeds for 1, 2 and 3 min, followed by thorough washing to remove the acid. iii) for Manual Scratching, seeds were manually scarified using abrasive material like scratch paper to physically disrupt the seed coat, facilitating water imbibition and gas exchange. iv) for Hot Water Treatment, seeds were subjected to a hot water bath for 2, 4 and 8 hr, then allowed to cool gradually to room temperature, to soften the seed coat. v) for Gibberellic Acid (GA_3) Application, seeds were soaked in solutions of gibberellic acid at concentrations of 250, 500 and 1000 ppm for 24 hr in dark conditions to evaluate the hormonal effect on dormancy breaking. Control group was maintained where seeds were not subjected to any dormancy-breaking treatment to establish a baseline germination rate under natural conditions. Post-treatment, seeds were placed on moist filter paper in Petri dishes and incubated at 25 °C with a 12 hr photoperiod, simulating optimal germination conditions (14). Germination was monitored daily for 14 days, with germination defined as the emergence of the radicle. The germination rate for each treatment was calculated as the percentage of seeds that successfully germinated, compared to the total number of seeds in each group. Statistical analysis was employed to determine the significance of differences in germination rates between treatments and the control group. The primary objective of this experimental design was to identify the most effective dormancy-breaking treatment for *S. portulacastrum* seeds, taking into consideration both the germination rates and the practicality of each method for broader application in conservation and agriculture.

Pre-treatment of Seeds

Potassium Nitrate Treatment

Potassium nitrate (KNO_3) is a well-known chemical treatment used to break seed dormancy and promote germination in various plant species. The effectiveness of potassium nitrate is primarily due to its role in enhancing the nitrate availability, which is a critical signaling molecule involved in seed germination (15, 16). Nitrate is thought to act as both a nutrient and a signaling molecule, which can trigger a cascade of biochemical processes that lead to the breaking of dormancy and initiation of germination.

In this study, *Sesuvium portulacastrum* seeds were subjected to 2% potassium nitrate solution to assess its efficacy in breaking seed dormancy. The choice of concentration was based on previous studies (17) have demonstrated its effectiveness in improving germination rates in other species with similar dormancy mechanisms.

For the potassium nitrate treatment, seeds were first thoroughly rinsed with distilled water to remove any residual bleach. Then 25 number of seeds were then soaked in a freshly prepared 2% potassium nitrate (KNO_3) solution for 10 min, 15 min and 24 hr separately at room temperature. This soaking period was selected to allow sufficient time for the nitrate ions to penetrate the seed coat and reach the embryo, thus initiating the dormancy-breaking process (18). After soaking, the seeds were removed from the solution, rinsed briefly with distilled water and placed on moist filter paper in Petri dishes. The Petri dishes were then incubated in a controlled environment at 25 °C, with light conditions mimicking a 12 hr photoperiod, which is optimal for the germination of many halophytes (19). The germination process was monitored daily for up to 14 days and the germination rate was recorded as the percentage of seeds that successfully developed a radicle.

Sulfuric Acid (H_2SO_4) Treatment

Sulfuric acid (H_2SO_4) is a widely used chemical scarification agent for breaking seed dormancy, particularly in seeds with hard, impermeable seed coats. The use of concentrated sulfuric acid is effective in etching the seed coat, which facilitates water imbibition and gas exchange, both critical for the initiation of germination (20). To evaluate the efficacy of sulfuric acid, we followed methods established (13). Briefly, 25 number of seeds were placed in a concentrated sulfuric acid treatment for 1, 2 and 3 min under controlled conditions (under Laboratory hood). After applying the treatment, the seeds were washed with distilled water. After washing, the seeds were kept at ambient temperature (27 ± 2) for 3 hr and after drying, the seeds were placed on moist germination paper for 14 days to assess germination rate and seedling parameters in Petri dishes. The dishes were incubated at 25 °C under a 12 hr photoperiod and germination was monitored daily for 14 days.

Manual Scratching

Physically abrading the seed coat to enable water and gas exchange necessary for germination. This is achieved by gently scratching the seed's surface with abrasive material like scratch paper taking care not to damage the embryo within. The objective is to lightly wear away part of the hard outer coating until a noticeable thinning or small opening is created (21) and reinforced by the comprehensive work on seed dormancy (22), highlights mechanical scarification as an effective dormancy-breaking technique, especially for species with notably hard seed coats, thereby facilitating the initiation of the germination process (22). This hands-on approach serves as a direct and controlled method to overcome one of the primary physical barriers to seed germination, offering a practical solution for propagating difficult-to-germinate species. Briefly, 25 seeds were subjected to manual scratching using fine-grade sandpaper for durations of 1, 2 and 3 min under controlled laboratory conditions. After that the seeds were placed on moist germination paper in Petri dishes. The Petri dishes were incubated at a constant temperature of 25 °C under a 12 hr photoperiod and germination was monitored daily for 14 days.

Hot Water Treatment

Hot water treatment is a commonly used physical method to break seed dormancy, especially in seeds with hard or impermeable seed coats. This method involves exposing seeds to high temperatures for a short duration, which helps to soften the seed coat and promote water uptake, thus facilitating germination (12). To evaluate the efficacy of hot water, we used methods described (23) where *Sesuvium portulacastrum* seeds were placed in a hot water bath (hot water) for 2 hr, 4 hr and 8 hr. After the immersion, the seeds were allowed to cool gradually in the same water as it returned to room temperature. This gradual cooling is important to avoid thermal shock, which can damage the seed embryo (24). After the 2, 4 and 8 hr, the seeds were placed at an ambient temperature of 27 ± 2 °C for 3 hr to dry. After drying, 25 number of seeds were placed on moist filter paper in Petri dishes. The dishes were incubated at 25 °C under a 12 hr photoperiod, and germination was monitored daily for 14 days. The hot water treatment resulted in a modest increase in germination rate, with 22% of the treated seeds germinating, compared to 4% in untreated seeds.

The mechanism by which hot water treatment breaks dormancy involves the heat-induced softening of the seed coat, which increases permeability to water and gases, essential for germination. While effective, this method is generally less aggressive than chemical treatments like sulfuric acid and is therefore better suited for seeds that are sensitive to more invasive treatments (25). Hot water treatment has been successfully used in a variety of species to break dormancy. For example, it was found that hot water treatment significantly increased germination rates in legume species (26). Similarly, it was demonstrated the effectiveness of hot water treatment in enhancing germination in seeds with hard coats (27).

Application of gibberellic acid

To evaluate the efficacy of the known dormancy-breaking hormone gibberellic acid (28), *Sesuvium portulacastrum* seeds were placed in different concentrations of 250 ppm, 500 ppm and 1000 ppm in the dark at 27 ± 2 °C. After the defined time, the seeds were removed from the gibberellic acid container and washed with distilled water based on the protocols (14). After the washing process, the seeds were kept at ambient temperature (27 ± 2) for 3 hr and after drying, 25 number of seeds were placed on moist filter paper in Petri dishes and in a growth chamber at 25 °C with a 12 hr photoperiod. Germination was monitored daily for 14 days and seeds were considered germinated when the radicle protruded.

Germination percentage

Results of comparing the average seed germination percentage was calculated in Table 1 according to Equation (1) described (14).

$$GP = S/T \times 100 \quad \text{……...(Eqn. 1)}$$

Table 1. Results of comparing the average germination

Sl. No	Treatment	Treatment condition	Germination percentage (%)
1.	Control	-	4
2.	KNO_3, 2%	10 min	16
		15 min	8
		Overnight	12
3.	H_2SO_4, Concentrated	1 min	72
		2 min	88
		3 min	42
4.	Hot water	2 hr	20
		4 hr	22
		8 hr	16
5.	Manual scarification	1min	8
		2 min	7
		3 min	0
6.	Gibberellic acid	250 ppm	0
		500 ppm	0
		1000 ppm	0

Statistical Analysis

Germination percentage was calculated for each treatment and the data were analyzed using ANOVA (Table 2). The ANOVA is based on a completely randomized design (CRD) with 3 replication to determine the significance of differences between treatments

Results

The germination results revealed significant differences among the treatments ($p < 0.05$). Sulfuric acid treatment for 2 min yielded the highest germination rate of 88%, significantly outperforming all other treatments (Fig. 3). Potassium nitrate and hot water treatments resulted in

Table 2. Results of analysis of variance for the effect of different methods of break seed dormancy on some germination characteristics of *Sesuvium portulacastrum* seeds

SV	DF	SS	MSS	FCAL	FTABLE		RESULT
					at 5 per	at 1 per	
TRT	14	29801.24	2128.66	818.7155	2.03742	2.741805	S
A	4	26257.24	6564.311	2524.735	2.689628	4.017877	S
B	2	999.5111	499.7556	192.2137	3.31583	5.390346	S
AB	8	2544.489	318.0611	122.3312	2.266163	3.172624	S
ERROR	30	78.00	2.6				
TOTAL	44	29879.24					

Fig. 3. Germination of *Sesuvium portulacastrum* seeds after sulfuric acid treatment.

moderate germination rates of 18% and 20% respectively. Manual scratching and Gibberellic acid treatment were less effective, with germination rates below 8% mentioned (Fig. 4).

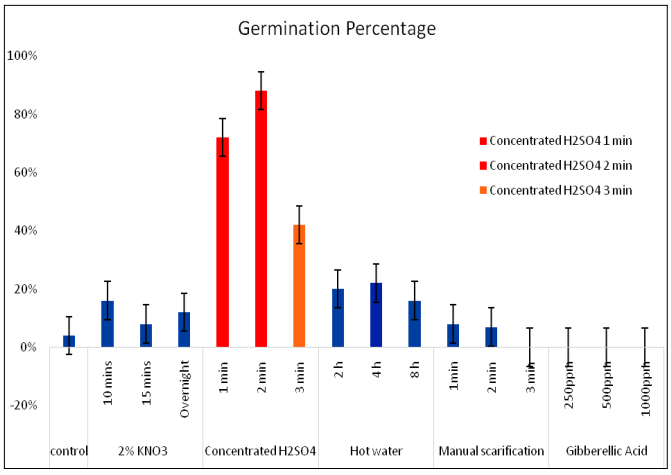

Fig. 4. Effect of various treatments on *Sesuvium portulacastrum* seed 14 days after no treatment (Control), application of 2% KNO_3 treatment for 10, 15 min and overnight, sulfuric acid for 1, 2 and 3 min, pretreatment with hot water for 2, 4 and 8 min, manual scarification for 1, 2 and 3 min, gibberellic acid at 250, 500 and 1000 parts per million. Twenty-five seeds were used for each technical replicate. Data shown represent averages and standard deviation for 3 technical replicates for each treatment.

The germination results varied significantly across treatments. The control exhibited a minimal germination rate of 4%, highlighting the presence of dormancy barriers (Fig. 5). Among the treatments, sulfuric acid (H_2SO_4) was the most effective, with a 2 min exposure achieving the highest germination rate of 88% (Fig. 3), followed by 1 min

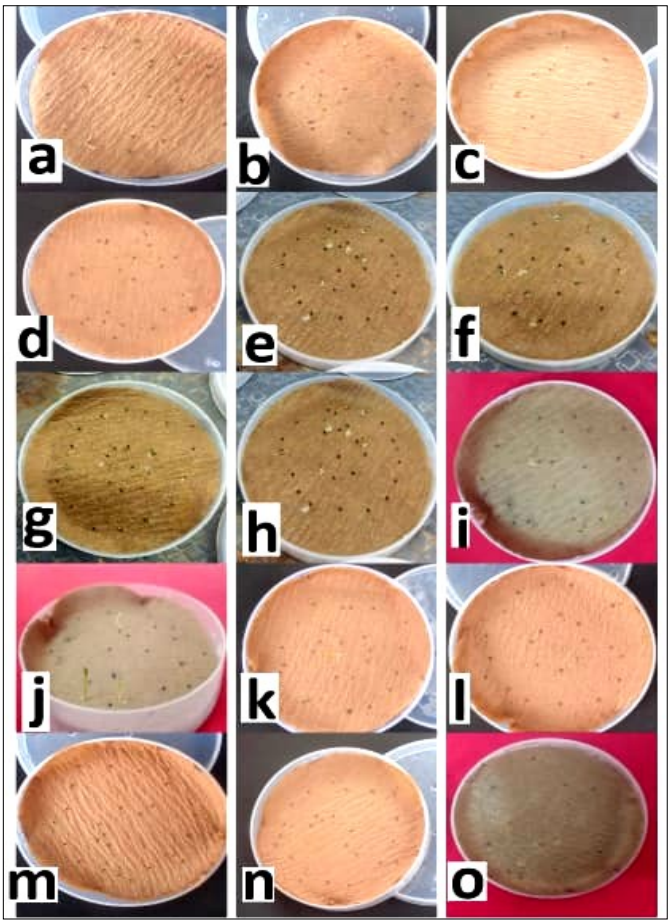

Fig. 5. Germination percentage of *Sesuvium portulacastrum* seeds after various treatment. **a**- control, **b**- 2% KNO_3 for 10 min, **c**- 2% KNO_3 for 15 min, **d**- 2% KNO_3 for overnight, **e**- Concentrated H_2SO_4 for 1 min, **f**- Concentrated H_2SO_4 for 3 min, **g**- Hot water treatment for 2 hr, **h**- Hot water treatment for 4 hr, **i**- Hot water treatment for 8 hr, **j**- Manual scarification for 1 min, **k**- Manual scarification for 2 min, **l**- Manual scarification for 3 min, **m**- Gibberellic acid treatment at 250 ppm, **n**- Gibberellic acid treatment at 500 ppm, **o**- Gibberellic acid treatment at 1000 ppm.

at 72%, while 3 min reduced germination to 42% due to potential seed damage (Fig. 5). Potassium nitrate (KNO_3) treatments showed moderate success, with a 10 min exposure yielding 16% (Fig. 5), while overnight and 15 min exposures resulted in 12% and 8% respectively. Hot water treatments produced moderate germination rates of 20% for 2 hr, 22% for 4 hr and 16% for 8 hr, indicating diminishing returns with prolonged exposure (Fig. 5). Manual scarification had limited effectiveness, with rates of 8% and 7% for 1 and 2 min respectively, while 3 min completely inhibited germination (Fig. 5). Gibberellic acid (GA_3) at concentrations of 250 ppm, 500 ppm and 1000 ppm showed no germination (Fig. 5), underscoring its ineffectiveness in addressing the physical dormancy of *Sesuvium portulacastrum* seeds.

These results emphasize the importance of sulfuric acid treatment for breaking physical dormancy and achieving optimal germination rates.

Discussion

This study undertook a comprehensive analysis to compare various dormancy-breaking methods for *Sesuvium portulacastrum* seeds, with a focus on sulfuric acid treatment due to its significant implication in enhancing germination rates. The findings from this investigation offer pivotal insights into the most effective methods for overcoming seed dormancy in *S. portulacastrum*, highlighting sulfuric acid treatment as the superior approach among those tested, including potassium nitrate, manual scratching, hot water and gibberellic acid treatments.

Sulfuric acid's efficacy in breaking seed dormancy aligns with the observations (29), where the application of sulfuric acid was noted to significantly improve germination rates across species with hard seed coats. The mechanism behind this success seems to be sulfuric acid's ability to penetrate and dissolve the physical barriers posed by the seed coat effectively, thus facilitating water uptake and gas exchange (30). This study corroborates these findings, noting a substantial increase in germination rates with sulfuric acid treatment for 2 min, suggesting an optimal balance between duration of exposure and concentration that safely yet effectively weakens the seed coat without causing detrimental effects to the seed integrity or germination potential.

Contrastingly, other methods such as potassium nitrate treatment, although beneficial in enhancing nitrate availability which is crucial for germination as supported (31), did not yield as significant improvements in germination rates as sulfuric acid treatment. These findings suggest that while chemical signals like nitrate are essential for seed dormancy breakage, overcoming physical dormancy barriers with sulfuric acid is more critical for *S. portulacastrum* seeds (32).

Manual scratching and hot water treatments, despite being less aggressive and safer methods, provided moderate improvements in germination rates. These methods' efficacy, as discussed (33), underscores the importance of mechanical manipulation and thermal treatments in altering seed coat integrity to promote water uptake. However, their comparative suboptimal performance underscores the necessity of stronger interventions like sulfuric acid treatment for seeds with notably hard coats.

Furthermore, the application of gibberellic acid, a known dormancy-breaking hormone, demonstrated its potential in promoting germination, aligning with another work (34). Despite its physiological importance in overcoming dormancy, its effectiveness was surpassed by the chemical scarification method using sulfuric acid, suggesting that addressing the physical constraints to water and gas exchange takes precedence over purely hormonal interventions for *S. portulacastrum* (35).

The implications of these findings are substantial for the conservation and agricultural utilization of *S. portulacastrum*. Successfully breaking seed dormancy at higher rates not only facilitates restoration projects in saline and arid environments but also opens new avenues for the species' use in phytoremediation and as a sustainable crop in salt-affected areas. This research contributes to the broader understanding of plant adaptation and germination ecology, highlighting the need for targeted dormancy-breaking techniques to leverage the full potential of resilient plant species like *S. portulacastrum* in environmental management and agriculture (36).

The merits of this study lie in its direct comparison of various dormancy-breaking methods under controlled conditions, providing clear evidence of sulfuric acid's superiority. Unlike previous studies that often focused on a single treatment or generalized conclusions across species, this research specifically addresses the unique challenges posed by *S. portulacastrum* seeds. By doing so, it highlights sulfuric acid treatment's unparalleled efficiency in achieving high germination rates, a finding that could inform large-scale propagation efforts. Moreover, this study's practical applications in ecological restoration and sustainable agriculture underscore its relevance and utility.

In comparison to previous studies on similar halophytic species, our findings emphasize the critical role of physical dormancy-breaking techniques over hormonal or chemical signaling treatments alone. For instance, while (37) reported moderate success with hormonal treatments in other halophytes, this study's results clearly demonstrate that the physical barriers of *S. portulacastrum* seeds necessitate more robust approaches. Future research should focus on optimizing sulfuric acid treatment for varying environmental conditions and assessing its long-term impacts on seedling establishment and ecosystem resilience. These steps would ensure that the high germination rates observed translate into successful plant growth and ecosystem benefits.

Conclusion

The present study has effectively demonstrated that sulfuric acid treatment surpasses other dormancy-breaking methods, such as potassium nitrate application, manual scratching, hot water treatment and gibberellic acid exposure, in promoting germination in *Sesuvium portulacastrum* seeds. This finding is pivotal, given the ecological significance and potential applications of *S. portulacastrum* in environmental restoration and agriculture, particularly within saline-affected and arid regions. Sulfuric acid's proficiency in enhancing germination rates by weakening the physical barriers of the seed coat offers a practical and efficient approach for overcoming seed dormancy in *S. portulacastrum*. While this method requires careful handling due to the corrosive nature of sulfuric acid, the considerable improvement in germination rates justifies its application, aligning with previous research that highlights the efficacy of chemical scarification methods.

The findings from this study contribute significantly to the understanding of seed dormancy mechanisms in halophytic species and the optimization of germination protocols, offering valuable insights for conservationists, restoration ecologists and agricultural practitioners aiming to utilize *S. portulacastrum* for environmental and agricultural benefits. Future research should aim at refining sulfuric acid treatment protocols to maximize germination success while ensuring the safety and viability of the seeds. Additionally, exploring the long-term effects of enhanced germination on plant growth, survival and ecological integration within restoration projects would provide valuable information for the sustainable use of *S. portulacastrum* in combating desertification, managing soil salinity and contributing to biodiversity in saline ecosystems. In summary, the comparative analysis of seed dormancy-breaking methods in this study underscores sulfuric acid treatment as the most effective approach for *S. portulacastrum*, setting a foundation for further research and application in ecological restoration and sustainable agricultural practices in challenging environmental conditions.

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Authors' contributions

Conceptualization, KBR, KGSRS; data analysis and curation, KGSRS, KBR; writing-original draft preparation, KBR, PD, KGSRS; software application, KBR, KGSRS; review and editing, KBR, PD, VM, JD; visualization, KG. SRS, KBR; methodology, KBR, KGSRS, PD; Resources, KBR; Project Principal Investigator, PD. All co-authors read and agreed the final version and approved the manuscript before submission.

Compliance with ethical standards

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Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT in order to check grammar and improve readability. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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