

Article

Seed Priming as a Tool for Ex Situ Conservation: Improving Micropropagation and Acclimatization Success of the Balkan Endemic *Silene sendtneri* Boiss

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Abstract

This study evaluated the effects of different seed priming treatments on the ex situ propagation of the Balkan endemic species *Silene sendtneri* Boiss. Seeds were subjected to hydropriming, silicic acid, salicylic acid, proline and combined salicylic acid–proline treatments prior to in vitro cultivation. Following aseptic germination, seedlings were transferred to Murashige and Skoog medium supplemented with 1.0 mg L^{−1} BA and 0.2 mg L^{−1} IBA, and their shoot multiplication response, rooting, and acclimatization success were assessed. Seed priming significantly influenced subsequent propagation stages. Plantlets derived from seeds treated with 0.5 mM salicylic acid and 10 mM proline + 0.5 mM salicylic acid exhibited the highest multiplication rates, superior morphological quality, increased rooting frequency, and the greatest acclimatization success. Hydropriming and higher proline concentrations generally resulted in reduced propagation performance. The results indicate that seed priming can influence multiple stages of the ex situ propagation process and may represent a simple and practical addition to micropropagation protocols aimed at improving conservation-oriented plant production of endemic species.

Keywords: endemic plants; seed priming; plant conservation; conservation biotechnology; propagation efficiency; restoration

1. Introduction

Biodiversity loss is accelerating worldwide and represents one of the most significant environmental challenges of the twenty-first century. Habitat degradation, land-use change, climate change, invasive species, and other anthropogenic pressures are causing unprecedented declines in species diversity and ecosystem integrity [1]. According to recent global assessments, nearly one million species are currently threatened with extinction, emphasizing the urgent need for effective and innovative conservation strategies [1]. Endemic plant species are particularly vulnerable because of their restricted geographic distributions, fragmented populations, limited dispersal capacity, and specialized ecological requirements [2,3].

To address these challenges, conservation efforts increasingly combine in situ and ex situ approaches. While in situ conservation remains essential for maintaining ecological interactions and evolutionary processes, ex situ conservation provides an important complementary strategy for safeguarding threatened taxa, preserving genetic diversity, and supporting future restoration and reintroduction programs [4–7]. The importance of



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strengthening plant conservation efforts has been further recognized through international initiatives such as the Kunming–Montreal Global Biodiversity Framework and the European Union Biodiversity Strategy for 2030, both of which emphasize the urgent need to prevent biodiversity loss and improve species recovery actions [8,9].

Among available ex situ conservation tools, plant tissue culture and micropropagation have become increasingly valuable for the conservation of rare, endemic, and threatened plant species [10–14]. Micropropagation enables rapid multiplication from limited sources of material, reduces collection pressure on natural populations, facilitates long-term maintenance of valuable genotypes, and generates plant material for research, restoration, and reinforcement programs [10–13]. Consequently, conservation biotechnology is now recognized as an important component of integrated plant conservation strategies, particularly for species with limited reproductive capacity, poor seed germination, or fragmented populations [13,15–17].

Despite its considerable potential, the successful application of micropropagation in conservation programs remains constrained by several developmental bottlenecks. Efficient culture establishment, shoot multiplication, rooting, and especially acclimatization to ex vitro conditions are critical determinants of propagation success [11,14]. Acclimatization is frequently associated with substantial plant losses due to abrupt changes in humidity, irradiance, temperature, and nutrient availability following transfer from controlled in vitro conditions [11,14]. Improving plant performance throughout the entire propagation pipeline therefore remains a priority in conservation-oriented propagation systems.

One potentially valuable but still underutilized approach is seed priming. Seed priming is a pre-sowing treatment involving controlled hydration that initiates metabolic processes associated with germination while preventing radicle emergence [18]. Extensive research conducted on agricultural species has demonstrated that priming can improve germination performance, seedling establishment, plant vigor, and tolerance to environmental stress [18–20]. Various priming agents, including osmoprotectants, signaling molecules, and chemical elicitors, have been shown to induce physiological and biochemical changes that enhance subsequent plant performance [18–20].

Increasing evidence suggests that priming-induced responses may persist beyond germination and influence later developmental stages [18,21]. Such carry-over effects indicate that physiological modifications initiated during the seed stage may affect subsequent growth, morphogenesis, and plant establishment. However, while seed priming has been extensively investigated in crop production systems, its application within plant conservation remains poorly explored. In particular, little attention has been devoted to understanding whether seed priming can improve subsequent stages of ex situ conservation pipelines, including in vitro establishment, shoot multiplication, rooting efficiency, and acclimatization success. Such improvements could substantially increase the effectiveness of conservation programs by enhancing both the quantity and quality of regenerated plant material.

Seed priming has previously been demonstrated to enhance germination performance, seedling establishment, and tolerance to cadmium stress in *Silene sendtneri*. In particular, priming with silicic acid, proline, and salicylic acid significantly improved germination, root growth, seedling development, and physiological performance under Cd stress, while also enhancing cadmium accumulation and stress tolerance [22]. These findings suggested that seed priming represents a promising strategy for improving the establishment and resilience of this endemic species. However, whether the beneficial effects induced during seed germination persist throughout subsequent stages of ex situ propagation, including shoot multiplication, rooting, and acclimatization, has remained unknown.

The Balkan Peninsula represents one of Europe's most important biodiversity hotspots and harbors a large number of endemic plant species adapted to geographically restricted and ecologically specialized habitats [2]. Among these species, *Silene sendtneri* Boiss. (Caryophyllaceae) is a Balkan endemic taxon associated with calcareous and serpentine substrates and characterized by a fragmented distribution within the western Balkans [22–25]. Owing to its restricted occurrence and ecological specialization, the development of effective ex situ conservation strategies is particularly important for safeguarding its genetic resources and supporting future conservation actions.

The objective of the present study was to determine whether the beneficial effects of seed priming extend beyond germination and influence subsequent stages of the ex situ propagation pipeline of *Silene sendtneri*. Specifically, we evaluated the effects of different priming treatments on shoot proliferation, rooting frequency, morphological quality, and acclimatization success of in vitro-derived plantlets, with the aim of developing a more efficient propagation protocol for the conservation of this endemic species.

2. Materials and Methods

2.1. Plant Material and Seed Priming Treatments

Seeds of the *Silene sendtneri* species were collected in July 2024 from a natural population located in the vicinity of Pjeskovita Ravan, Mt. Ozren, Bosnia and Herzegovina (43°54'19.1" N, 18°27'17.0" E; 1302 m a.s.l.). To minimize disturbance to the natural population, only mature seeds were collected, while all other plant material remained undisturbed. Following collection, seeds were transported to the Laboratory for Plant Physiology and Molecular Biology, University of Sarajevo, where they were stored at 4 °C until use. A total of 330 seeds were used in the experiment (30 seeds per treatment across the eleven experimental treatments). The experiment was conducted using three independent biological replicates, each consisting of ten seeds per treatment. Healthy seedlings obtained from each biological replicate were used for multiplication and subsequently evaluated.

Seed priming was performed by submerging seeds for 24 h at 4 °C in one of the following solutions: distilled water (hydropriming), 1% silicic acid (SiA), 0.5 mM salicylic acid (SA0.5), 1.0 mM salicylic acid (SA1), 10 mM proline (Pro10), 20 mM proline (Pro20), 0.5 mM salicylic acid + 10 mM proline (SA0.5+Pro10), 0.5 mM salicylic acid + 20 mM proline (SA0.5+Pro20), 1.0 mM salicylic acid + 10 mM proline (SA1+Pro10), and 1.0 mM salicylic acid + 20 mM proline (SA1+Pro20). Non-primed seeds served as the control treatment.

Following priming, seeds were rinsed twice with sterile distilled water and air-dried for 72 h to restore their initial moisture content. Dried seeds were subsequently stored in sterile tubes containing silica gel and maintained at 4 °C until further use. The selected priming agents were chosen based on their previously reported roles in improving seed performance and plant establishment [18–21].

2.2. In Vitro Germination and Culture Establishment

Primed and non-primed seeds were surface-sterilized by immersion in 70% (v/v) ethanol (Sigma-Aldrich Inc. (3050 Spruce Street, St. Louis, MO 63103, USA)) for 30 s, followed by 20% (v/v) commercial sodium hypochlorite solution containing a few drops of Tween-20 (Croda International Plc, Snaith, UK) for 15 min. Seeds were subsequently rinsed three times with sterile distilled water before being cultured aseptically on hormone-free Murashige and Skoog (MS) medium (HiMedia Laboratories, Mumbai, India) supplemented with MS vitamins, 3% (w/v) sucrose, and 8 g L⁻¹ agar (Duchefa Biochemie, Haarlem, The Netherlands). The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C and 103 kPa for 20 min. Approximately 25 mL of medium was dispensed into sterile glass culture vessels (100 mL capacity). Cultures were maintained in a growth chamber under

controlled environmental conditions consisting of a 16 h photoperiod provided by cool-white fluorescent lamps (2000 lux), a temperature of 23 ± 2 °C, and relative humidity of approximately 70%. After four weeks of cultivation, germinated seedlings were evaluated and used as source material for shoot multiplication.

2.3. Shoot Multiplication

To investigate whether seed priming influences subsequent stages of ex situ propagation, seedlings obtained from the different priming treatments were transferred to multiplication medium. Prior to transfer, roots were removed from shoots in order to standardize explant material and ensure that subsequent growth responses reflected shoot regeneration capacity rather than differences in root development during germination.

Uniform shoot explants consisting of the shoot apex with the basal stem portion (following complete removal of the root system) were transferred to the Murashige and Skoog (MS) basal medium supplemented with 7 g L⁻¹ agar, 1.0 mg L⁻¹ 6-benzyladenine (BA; HiMedia Laboratories, Mumbai, India) and 0.2 mg L⁻¹ indole-3-butyric acid (IBA; HiMedia Laboratories, Mumbai, India). This growth regulator combination was selected based on preliminary optimization experiments that demonstrated superior shoot proliferation, plant vigor, and culture stability compared with other tested media formulations. Cultures were maintained under the same environmental conditions described above. After four weeks of cultivation, shoot number per explant and overall morphological quality were evaluated.

The Morphological Quality Index (MQI) was developed for this study based on visual criteria commonly employed for the assessment of plantlet quality in micropropagation studies (Table 1). Thirty plantlets per treatment (three biological replicates of ten explants each) were evaluated for MQI.

Table 1. Plantlets were assigned a score from 1 to 5 according to the following criteria.

Score	Description
1	Very poor quality; severe chlorosis and/or necrosis, strongly reduced growth, malformed shoots
2	Poor quality; weak growth, pronounced leaf deformation or discoloration
3	Moderate quality; acceptable growth with minor morphological abnormalities
4	Good quality; vigorous growth, well-developed leaves, compact rosette morphology
5	Excellent quality; highly vigorous shoots, compact and symmetrical rosettes, healthy dark-green foliage, no visible signs of physiological disorders

The final morphological quality score for each treatment was calculated as the mean value obtained from all evaluated plantlets.

2.4. Rooting and Acclimatization

During the multiplication phase, a proportion of regenerated shoots developed roots during the multiplication phase. Rooting frequency was calculated as the percentage of explants that developed visible roots during the multiplication phase. Plantlets exhibiting healthy shoots and a functional root system were selected for acclimatization.

Rooted plantlets were carefully removed from culture vessels and residual agar was gently washed from the roots using distilled water. Plantlets were transplanted into 7 cm diameter plastic pots arranged in plastic trays containing a sterile substrate mixture consisting of commercial peat-based potting soil and perlite (3:1, v/v) because of its favorable water-holding capacity and aeration properties.

Immediately after transplantation, plants were maintained under high-humidity conditions by covering the trays with transparent polyethylene film. During the first week, humidity was maintained close to saturation to reduce transpirational stress. The trays were covered with transparent polyethylene film (commercial food-grade polyethylene film,

Pehart, Bosnia and Herzegovina). The humidity was gradually reduced over a two-week period by progressively opening the covers, allowing plants to adapt to ambient conditions.

Plants were maintained at temperatures ranging from 22 to 25 °C under natural daylight conditions and watered regularly to maintain adequate substrate moisture. Acclimatization success was evaluated after 45 days as the percentage of surviving plants in each biological replicate. Survival percentages were calculated separately for each of the three biological replicates and are presented as mean $\pm$ SD. Plant vigor, leaf development, and the occurrence of morphological abnormalities were also recorded. Rosette area was determined from overhead digital photographs using ImageJ software v1.51 (National Institutes of Health, Bethesda, MD, USA). The projected rosette area (cm²) of each plant was calculated after calibration using a reference scale included in each image.

2.5. Experimental Design and Statistical Analysis

The experiment included eleven seed treatments: one non-primed control and ten priming treatments. Each treatment consisted of three independent biological replicates, with ten plants evaluated per replicate (30 plants per treatment in total). Data are presented as mean $\pm$ standard deviation (SD).

Statistical analyses were performed using STATISTICA 10.0 (StatSoft Inc., Tulsa, OK, USA). Prior to analysis, data were examined for compliance with the assumptions of normality and homogeneity of variances. Differences among treatments were evaluated using one-way analysis of variance (ANOVA). When significant treatment effects were detected, means were separated using Tukey's Honestly Significant Difference (HSD) post hoc test. Statistical significance was accepted at $p < 0.05$. Rooting frequency and acclimatization survival were calculated as the percentage of plants exhibiting root formation or surviving acclimatization, respectively, within each biological replicate (10 plants). Percentages were calculated separately for each of the three biological replicates and are presented as mean $\pm$ SD. Statistical comparisons among treatments were performed using one-way ANOVA followed by Tukey's HSD test. Percentage data were arcsine square-root transformed prior to statistical analysis to improve normality and homogeneity of variances. For clarity, untransformed percentage values are presented in the tables.

3. Results

3.1. Effect of Seed Priming on In Vitro Multiplication and Plant Quality

Seed priming significantly influenced the subsequent in vitro shoot multiplication response of *Silene sendtneri* (Table 2; Figure 1). Although all treatments produced viable cultures capable of shoot regeneration, substantial differences were observed in shoot proliferation, increased rooting frequency, and overall plant quality.

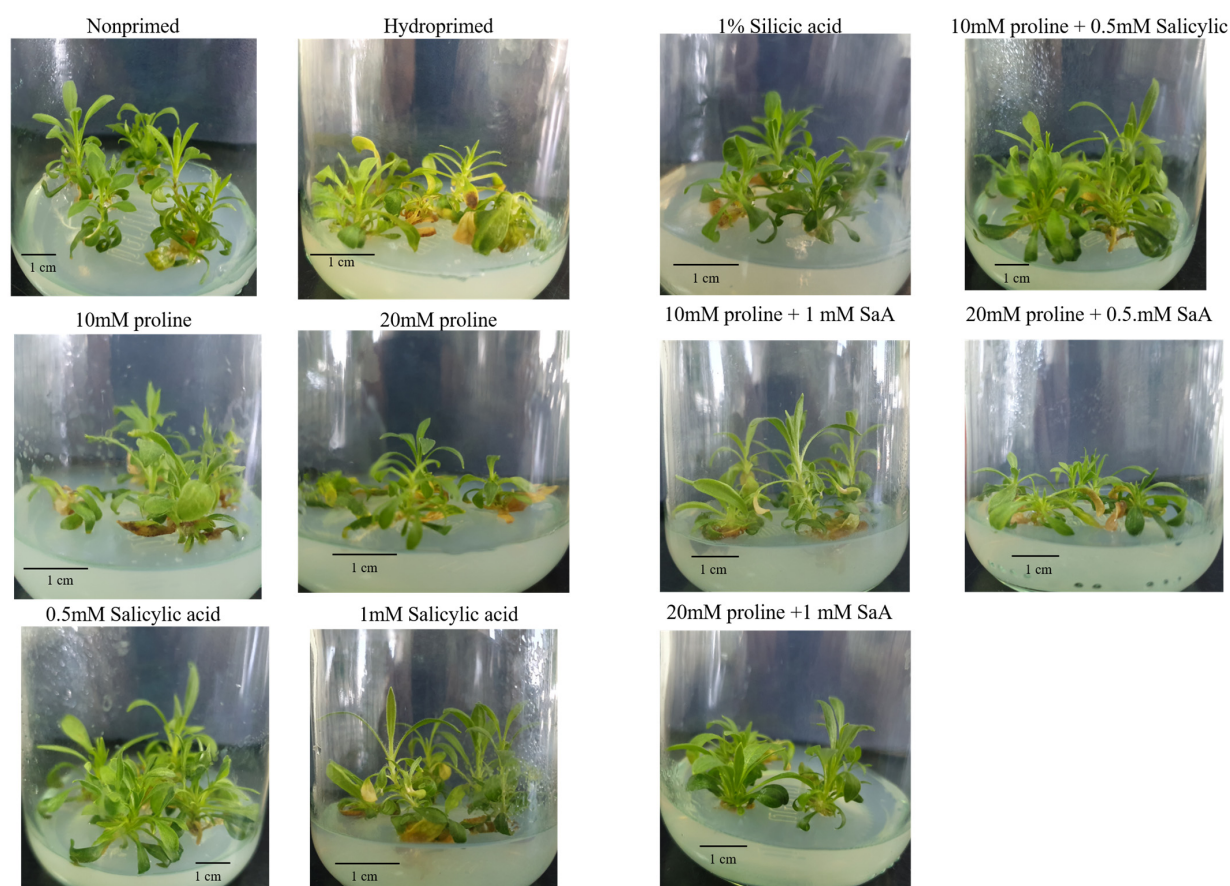
The highest multiplication response was obtained from seedlings originating from seeds primed with 10 mM proline + 0.5 mM salicylic acid, which achieved a multiplication response of 3.25 shoots per explant, representing a 44% increase compared with non-primed controls (2.25 shoots per explant). A similar response was observed in plantlets derived from seeds primed with 0.5 mM salicylic acid alone (2.75 shoots per explant). Both treatments also achieved the highest MQI (=5.0), indicating vigorous shoot growth, compact rosette formation, healthy leaf development, and the absence of visible necrosis.

Hydroprimed seedlings and plantlets derived from seeds treated with 20 mM proline exhibited the lowest multiplication response (1.25 shoots per explant) and reduced morphological quality. These cultures frequently developed smaller rosettes and showed slower overall growth. Increasing proline concentration from 10 to 20 mM generally resulted in reduced multiplication efficiency, suggesting a concentration-dependent effect of the priming treatment.

Table 2. Effect of seed priming treatments on in vitro multiplication, rooting frequency and morphological quality of *Silene sendtneri*.

Treatment	Multiplication Response	Rooting Frequency (%)	MQI
Non-primed	2.25 ± 0.50 ^c	50 ± 15 ^b	4.0 ± 0.5 ^b
Hydroprimed	1.25 ± 0.50 ^d	25 ± 10 ^c	2.5 ± 0.5 ^d
10 mM Proline	1.75 ± 0.50 ^d	50 ± 15 ^b	3.5 ± 0.5 ^c
20 mM Proline	1.25 ± 0.50 ^d	25 ± 10 ^c	3.0 ± 0.5 ^{cd}
0.5 mM SA	2.75 ± 0.50 ^b	75 ± 10 ^a	5.0 ± 0.0 ^a
1.0 mM SA	2.50 ± 0.50 ^b	50 ± 15 ^b	4.0 ± 0.5 ^b
1% Silicic acid	2.25 ± 0.50 ^c	50 ± 15 ^b	4.0 ± 0.5 ^b
10 mM Proline + 0.5 mM SA	3.25 ± 0.50 ^a	75 ± 10 ^a	5.0 ± 0.0 ^a
10 mM Proline + 1.0 mM SA	2.25 ± 0.50 ^c	50 ± 15 ^b	4.5 ± 0.5 ^{ab}
20 mM Proline + 0.5 mM SA	1.75 ± 0.50 ^d	50 ± 15 ^b	3.5 ± 0.5 ^c
20 mM Proline + 1.0 mM SA	1.50 ± 0.50 ^d	50 ± 15 ^b	3.5 ± 0.5 ^c

Values are presented as mean ± SD of three biological replicates (10 explants per replicate; $n = 30$ explants). Different letters within the same column indicate statistically significant differences among treatments according to one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) post hoc test ($p < 0.05$). MQI: Morphological Quality Index, scored on a scale from 1 (poor quality) to 5 (excellent quality).

**Figure 1.** In vitro regeneration of the endemic species *Silene sendtneri* following seed priming treatments used to support conservation-oriented propagation.

Seed priming also affected rooting frequency formation during the multiplication phase. The highest rooting frequencies (75%) were recorded in cultures originating from seeds treated with 0.5 mM salicylic acid alone and with 10 mM proline + 0.5 mM salicylic acid. In comparison, hydroprimed seedlings and cultures derived from 20 mM proline-treated seeds exhibited significantly lower rooting percentages (25%). Since plantlets with well-developed root systems can be transferred directly to acclimatization without an addi-

tional rooting phase, enhanced rooting frequency not only simplifies the propagation protocol but is also associated with improved morphological quality and subsequent acclimatization success, thereby increasing the overall efficiency of ex situ conservation programs.

3.2. Effect of Seed Priming on Ex Vitro Acclimatization Success

Substantial differences among treatments became evident during acclimatization (Figure 2, Table 3). While all treatments produced rooted plantlets capable of surviving transfer to substrate, acclimatization performance varied considerably depending on the seed priming treatment.

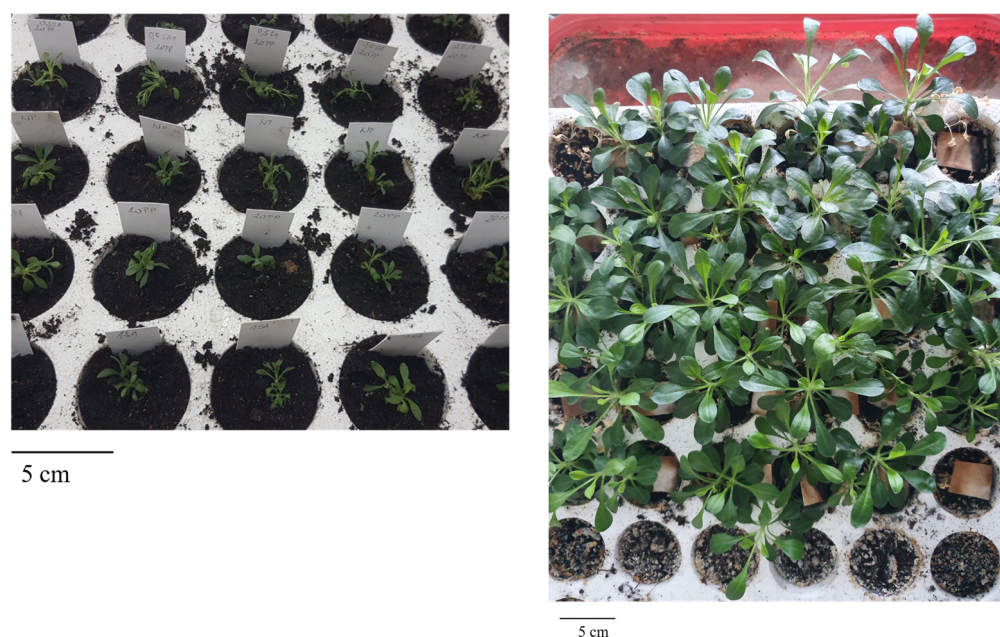


Figure 2. Acclimatization of the endemic species *Silene sendtneri* following multiplication (left start of the acclimatization process; right at the end of the process).

Table 3. Effect of seed priming treatments on acclimatization performance of *Silene sendtneri* after 45 days under ex vitro conditions.

Treatment	Survival (%)	Rosette Area (cm ²)
Non-primed	70 ± 12 ^{bc}	9.8 ± 1.5 ^{bc}
Hydroprimed	40 ± 10 ^e	5.2 ± 1.1 ^e
10 mM Proline	75 ± 10 ^{bc}	10.5 ± 1.6 ^{bc}
20 mM Proline	55 ± 11 ^d	7.4 ± 1.3 ^d
0.5 mM SA	95 ± 5 ^a	15.8 ± 2.2 ^a
1.0 mM SA	85 ± 8 ^{ab}	13.4 ± 1.8 ^{ab}
1% Silicic acid	82 ± 9 ^{ab}	12.8 ± 1.9 ^{ab}
10 mM Proline + 0.5 mM SA	100 ± 0 ^a	17.2 ± 2.4 ^a
10 mM Proline + 1.0 mM SA	88 ± 7 ^{ab}	14.0 ± 2.0 ^{ab}
20 mM Proline + 0.5 mM SA	72 ± 10 ^{bc}	10.2 ± 1.7 ^{bc}
20 mM Proline + 1.0 mM SA	68 ± 11 ^c	9.1 ± 1.5 ^c

Values are presented as mean ± SD of three biological replicates (10 explants per replicate; $n = 30$ explants). Different letters within the same column indicate statistically significant differences among treatments according to one-way ANOVA followed by Tukey's HSD post hoc test, $p < 0.05$.

The highest acclimatization success was recorded in plantlets originating from seeds primed with 10 mM proline + 0.5 mM salicylic acid, which achieved 100% survival and developed the largest rosettes (17.2 ± 2.4 cm²). Similarly, plantlets derived from seeds treated

with 0.5 mM salicylic acid exhibited 95% survival and large rosette areas ($15.8 \pm 2.2 \text{ cm}^2$), indicating excellent adaptation to ex vitro conditions.

Plantlets originating from seeds treated with 1.0 mM salicylic acid, 1% silicic acid, and 10 mM proline + 1.0 mM salicylic acid also performed well during acclimatization, with survival rates exceeding 80% and significantly greater rosette development compared with non-primed controls. In contrast, hydroprimed plantlets exhibited the lowest acclimatization success, with only 40% survival and the smallest rosette area ($5.2 \pm 1.1 \text{ cm}^2$). Reduced survival and growth were also observed in plantlets originating from seeds treated with 20 mM proline, suggesting that higher proline concentrations were less beneficial for post-transfer establishment.

Plantlets derived from salicylic acid-containing treatments developed compact dark-green rosettes and maintained active leaf production throughout the acclimatization period. Hydroprimed plantlets and those originating from 20 mM proline treatments frequently exhibited slower growth, reduced rosette expansion, and occasional leaf senescence.

Acclimatization performance closely reflected the responses observed during the multiplication phase. Treatments that produced higher multiplication indices and greater rooting frequencies generally exhibited superior survival and growth under ex vitro conditions. The consistency of these responses across propagation stages suggests that physiological effects induced during seed priming persisted beyond germination and continued to influence plant performance during acclimatization.

4. Discussion

The present study evaluated whether seed priming treatments applied prior to germination could influence subsequent stages of ex situ propagation in the Balkan endemic species *Silene sendtneri*. While the beneficial effects of seed priming on germination performance and stress tolerance have been extensively documented in agricultural species [18–21], comparatively little attention has been devoted to understanding whether these effects persist during later developmental stages relevant to plant conservation. The results obtained in this study suggest that seed priming may influence not only seed germination, as previously reported for *S. sendtneri* [22], but also subsequent in vitro multiplication, rooting frequency, and acclimatization performance.

In the present study, plantlets derived from seeds primed with salicylic acid, particularly in combination with 10 mM proline, generally exhibited superior shoot multiplication response, greater shoot proliferation, and improved morphological quality compared with non-primed controls. Although the physiological mechanisms underlying these responses were not investigated, previous studies have demonstrated that physiological changes induced during seed priming may persist beyond germination and influence subsequent developmental processes [18,21,24–26]. Such long-term responses have been associated with alterations in metabolic activity, antioxidant systems, stress signaling pathways, and hormonal regulation, potentially affecting growth and morphogenesis during later stages of plant development [18–20,24]. The results obtained here are consistent with the hypothesis that seed priming may generate a physiological state that continues to influence plant performance throughout subsequent stages of propagation.

The observed responses also indicate that priming intensity is likely to be an important factor influencing propagation outcomes. While treatments containing 10 mM proline generally performed well throughout the propagation process, increasing the concentration to 20 mM did not provide additional benefits and was frequently associated with reduced multiplication response and lower morphological quality. Similar concentration-dependent responses have been reported in seed priming studies where moderate treatment intensities produced more favorable outcomes than higher concentrations [19,20,25,26]. Such findings

emphasize the importance of species-specific optimization when developing conservation-oriented priming protocols.

The enhanced shoot proliferation observed following seed priming with 0.5 mM salicylic acid, particularly when combined with 10 mM proline, is consistent with the concept that physiological conditioning during seed imbibition can influence subsequent morphogenetic responses during in vitro culture. Previous studies have shown that seed priming can modify antioxidant capacity, hormonal homeostasis, and metabolic activity, resulting in improved growth and developmental performance long after germination [18–21,24–26]. Although relatively few studies have investigated these carry-over effects during tissue culture, increasing evidence suggests that physiological changes induced during seed priming may persist beyond germination and influence subsequent developmental processes. Similar observations have been reported in studies demonstrating the beneficial effects of seed priming on plant vigor and regeneration potential in vitro [13,18,21,24,27,28].

An interesting observation was the close association between rooting frequency and the Morphological Quality Index (MQI). The treatments that exhibited the highest rooting frequencies, namely 0.5 mM salicylic acid and 10 mM proline + 0.5 mM salicylic acid, also produced plantlets with the highest MQI scores, characterized by vigorous growth, compact rosettes, healthy dark-green foliage, and the absence of visible physiological disorders. This relationship suggests that enhanced root development may contribute to improved overall plantlet quality by increasing the capacity for water and nutrient uptake and supporting balanced shoot development. Salicylic acid has been reported to regulate antioxidant metabolism, hormonal signaling, and stress responses, while proline contributes to osmotic adjustment and protection of cellular structures under stress conditions. These physiological effects may collectively improve the developmental competence of regenerants, resulting in both enhanced rooting and superior morphological quality. Similar associations between plantlet vigor, rooting performance, and subsequent acclimatization success have been reported in micropropagation studies of several species, highlighting the importance of producing well-developed plantlets prior to transfer to ex vitro conditions. From a practical perspective, rooting may simplify propagation procedures by reducing the need for a separate rooting phase and additional growth regulator treatments. Such simplification can be particularly valuable in conservation programs where propagation capacity, infrastructure, and financial resources may be limited [10,11,29–31]. Although rooting was not the primary focus of this study, the observed differences among treatments suggest that seed priming may influence developmental processes extending beyond shoot multiplication.

An important finding of this study was the enhanced rooting frequency observed in plantlets originating from seeds primed with salicylic acid, either alone or in combination with 10 mM proline. Root formation during the multiplication phase represents a practical advantage because it eliminates the need for a separate rooting stage, thereby reducing labor, culture time, and the consumption of culture media and plant growth regulators. Such simplification is particularly valuable in conservation programs, where resources are often limited and the rapid production of healthy planting material is essential [10–14,29–31]. Similar rooting responses have been reported in micropropagation studies of other *Silene* species, including *Silene leucophylla*, *Silene schimperiana*, and *Silene fabaria* subsp. *domokina*, where efficient root formation contributed to successful acclimatization and highlighted the value of in vitro propagation for ex situ conservation [32–35]. The close association observed in the present study between rooting frequency and the Morphological Quality Index further supports the view that vigorous root development contributes to improved plantlet quality through enhanced water and nutrient uptake and balanced shoot development.

The beneficial effects of salicylic acid and proline are likely to involve multiple physiological processes. Salicylic acid functions as an important signaling molecule regulating

antioxidant defense, reactive oxygen species homeostasis, and hormonal interactions associated with plant growth and stress responses. Proline contributes to osmotic adjustment, membrane stabilization, and protection of cellular macromolecules during environmental stress. The combination of these two priming agents may therefore establish a more favorable physiological status that persists beyond germination, supporting both root initiation and vigorous shoot development under in vitro conditions. Although the underlying mechanisms were not directly investigated in the present study, our observations are consistent with previous reports describing long-lasting physiological effects of seed priming on plant development.

Differences among treatments became even more apparent during acclimatization. The transition from in vitro to ex vitro conditions is widely regarded as one of the most critical stages of the micropropagation process because regenerated plants must rapidly adjust from a highly controlled environment to conditions characterized by greater fluctuations in humidity, irradiance, temperature, and water availability [11,14,27,28]. Mortality during this stage frequently represents a major limitation of propagation protocols and may substantially reduce the number of successfully acclimatized plants [27,28]. In the present study, plantlets derived from salicylic acid-based treatments generally exhibited superior survival and more vigorous growth during acclimatization compared with non-primed controls and several other priming treatments. These observations suggest that physiological conditioning induced during seed priming may contribute to improved establishment following transfer to substrate. These observations further indicate that the benefits of seed priming extend beyond the in vitro phase. Plantlets exhibiting higher rooting frequency and superior morphological quality were also those with the greatest acclimatization success, suggesting that improvements achieved during early developmental stages can translate into greater ex vitro performance. For conservation programs relying on limited seed resources, this cumulative improvement across the propagation pipeline is of considerable practical significance because it increases the number of healthy plants ultimately available for long-term conservation, population reinforcement, and restoration.

Silene sendtneri is a Balkan endemic species associated with specialized habitats and fragmented populations [22–25]. Such species often require conservation approaches that maximize propagation efficiency while minimizing impacts on natural populations. The results presented here indicate that seed priming may provide a simple and inexpensive means of improving propagation outcomes without requiring substantial modifications to established tissue culture protocols. Because priming is applied at the seed stage, it can be readily integrated into existing ex situ conservation workflows and may represent a practical addition to current propagation strategies.

The broader implications of this study extend beyond *S. sendtneri*. Several endemic and threatened plant species experience propagation limitations that restrict their incorporation into active conservation programs. Seed priming represents a low-cost and easily transferable intervention that can be combined with established micropropagation techniques to improve plant production efficiency. The integration of these complementary approaches may contribute to broader conservation biotechnology efforts aimed at safeguarding plant diversity through ex situ measures [13,29,30].

Several limitations should be acknowledged. The study focused on a single endemic species and was conducted under controlled propagation conditions. Furthermore, the physiological and molecular mechanisms responsible for the observed responses were not investigated. Consequently, the extent to which similar responses occur in other conservation-priority taxa remains uncertain. Future studies should evaluate whether comparable benefits can be achieved across a wider range of endemic and threatened species and should investigate the physiological basis of the observed carry-over effects. Future

research should also assess whether improved acclimatization performance translates into enhanced survival following field establishment, population reinforcement, or reintroduction programs, which ultimately represent the primary objectives of many conservation initiatives [4,5,36].

The results suggest that seed priming may influence multiple stages of the ex situ propagation process in *Silene sendtneri*. While additional studies are needed to validate the broader applicability of this approach, the findings indicate that seed priming has potential as a practical component of conservation-oriented propagation protocols for endemic plant species.

5. Conclusions

Seed priming influenced both in vitro multiplication and ex vitro establishment of the Balkan endemic species *Silene sendtneri*. Treatments based on 0.5 mM salicylic acid, particularly in combination with 10 mM proline, consistently produced the highest multiplication response, rooting frequencies, and acclimatization success. These findings suggest that seed priming may represent a simple and practical addition to micropropagation protocols, improving the efficiency of ex situ propagation from limited seed resources. Further studies are needed to determine whether similar responses occur in other conservation-priority species and under restoration conditions.

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Conflicts of Interest: The authors declare no conflicts of interest.

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