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Asexual Propagation Techniques for ex Situ Conservation of Satiny Willow (*Salix pellita* Anderss.)

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***Abstract.* *Salix pellita* (satiny willow) is a state-threatened shrub willow species native to Minnesota, USA, valued for its ornamental potential and its ecological value. Habitat loss has reduced its natural distribution in the United States significantly, prompting the need for effective ex situ conservation strategies using horticultural propagation techniques, likely with limited stock materials. Our study evaluated several asexual propagation techniques to develop a standardized protocol for conserving *S. pellita*. In substrate evaluations, semihardwood stem cuttings resulted in improved rooting success in 100% perlite (100% rooting) compared with a 50/50 peat/perlite mix (73% rooting) and 100% peat (42% rooting). Cuttings grown in perlite also exhibited increases in root number and length. In the cutting type and auxin treatment experiment, single-node stem cuttings achieved > 80% rooting success regardless of plant growth regulator (auxin) application, whereas leaf cuttings exhibited reduced rooting success (32%–66%) and did not produce shoots. In the population-based rooting trial, plants derived from five geographically distinct populations revealed uniform and high rooting success (97%–100%), although some populations exhibited differences in the number of roots generated. This set of experiments has identified effective substrate choices and viable asexual propagation techniques for *S. pellita*, even when stock material is limited. A consistent rooting response across distinct populations may indicate species-level uniformity in inducing adventitious root formation on semihardwood stem cuttings, providing practical insights for horticultural conservation and broader management strategies applicable to this taxon and other at-risk plant species.**

Willows (*Salix* spp.) are ecologically vital and botanically diverse, comprising more than 400 species across temperate regions of the northern hemisphere (Argus 2007; Newsholme 2003). Their adaptability enables them to thrive in habitats from alpine tundra to riparian wetlands, where they contribute to soil stability, hydrological processes, and habitat structure (Argus 2007). Horticulturally, they are valued for their ornamental features, rapid rates of growth, and adaptability. Although historically significant for use in medicine and agriculture, their primary importance lies in their ecological functionality (Kuzovkina et al. 2007; Moerman 1998; Sneader 2000). One species of particular interest both ecologically and because of its horticultural value is *Salix pellita* (satiny willow; Salicaceae). *Salix pellita* is a large shrub or small tree that attains heights of 4 to 5 m and is named for the dense, white pubescence on the abaxial surface of its leaves (Smith 2008). This species occurs in dynamic and often harsh environments, including gravelly riverbanks and rocky lakeshores (Smith 2018). These habitats are increasingly threatened by anthropogenic pressures such as shoreline development, altered hydrology, and competition with invasive species (Smith 2018). With its limited and isolated populations, particularly in Minnesota, this species is vulnerable to extreme weather events, pest outbreaks, and other environmental stressors. Although conservation measures such as state-level threatened or endangered legal listings and land protection have been enacted, these efforts alone may not be sufficient to safeguard the species in the face of accelerating environmental change (Smith 2008). As climate change intensifies and human development continues to fragment natural landscapes, the preservation of biodiversity, particularly of narrowly distributed species, has become a forefront conservation priority.

The majority of the native range of *S. pellita* is found in Canada, but the taxon has a limited and declining presence in the northern United States. Its distribution includes increasingly rare occurrences in the states of Minnesota (Coffin and Pfannmuller 1988), Wisconsin (Chadde 1998), Michigan (Voss 1985), and Vermont (Thompson 1989), with more historical occurrences in Maine (Richards et al. 1983) and New Hampshire (Seymour 1969). Within the United States, the westernmost limit of *S. pellita* is in Minnesota, with populations limited to a few sites in the northeastern region of the state. The species is ecologically significant because of its role in riparian ecosystems, where it stabilizes riverbanks and lakeshores, and provides habitat for a wide range of animal species (Smith 2018). This species holds potential for horticultural use as a result of its outstanding foliage characteristics, including satiny, white abaxial surfaces and dark-green adaxial leaf surfaces.

Given the scattered population distribution and vulnerability of the species across its US range, conservation efforts are limited in accessible plant material and genetic diversity for preservation. This underscores the need for complementary strategies such as ex situ conservation, which may help with the long-term preservation of valuable genetic resources and may reduce pressure on wild populations through optimized asexual propagation techniques. In *Salix*, asexual propagation can be advantageous when seed propagation is impractical, as seeds are short-lived or recalcitrant, making long-term storage difficult (Dickmann and Kuzovkina 2014). In addition, asexual propagation is preferred over seed propagation when the goal is to preserve specific genotypes with desirable traits.

The conservation of unique and threatened plant genetic resources follows two primary approaches: in situ conservation, which focuses on safeguarding plant populations within their natural habitats, and ex situ conservation, which preserves these plant species outside of their native environments (Cohen et al. 1991). For the latter, asexual propagation techniques are important tools for mass cultivation of at-risk species and play a crucial role in the success of ex situ conservation efforts (Cohen et al. 1991).

In general, willows are easy to propagate because of the presence of preformed root primordia on stem nodes (Kuzovkina et al. 2007); however, some exceptions exist, including *Salix caprea* L. (Chmelar and Meusel 1986) and *Salix humilis* Marshall (Schrader and Miller 2024). Many factors affect the ability of a species to be propagated successfully, including the cutting type, growing substrate, as well as the form and concentration of growth regulators (Hassanein 2013; Yoon et al. 2021). Previous studies have shown that lower concentrations (1000 and 3000 ppm) of auxins, such as indole-3-butyric acid (IBA) and potassium (K)-IBA (potassium salt), are effective at promoting adventitious root formation and root growth in *Salix* species

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(Edson et al. 1995; Evans 2001). To the best of our knowledge, there is no published literature regarding the propagation of *S. pellita*. Given the limited availability of propagules from vulnerable and often inaccessible populations, ex situ conservation efforts must be efficient and strategic. Developing a data-driven asexual propagation protocol can streamline propagation efforts, maximize the use of collected material, and support the establishment of a genetically diverse living collection. Therefore, a targeted investigation into asexual propagation methods for *S. pellita* is essential to inform and advance ex situ conservation strategies.

Ex situ conservation efforts with *S. pellita* would be facilitated with the development of a standardized asexual propagation protocol. Therefore, we conducted a series of experiments to address the knowledge gap impeding this effort. We hypothesized that semihardwood stem cuttings, single-node cuttings, and leaf cuttings from various *S. pellita* populations would develop adventitious roots successfully after treatment with standard concentrations of auxin-class plant growth regulators ("auxin"), and that substrate type, cutting type, and source provenance may influence propagation efficiency significantly. We conducted three experiments to meet the following objectives: 1) evaluate the impact of soilless substrates on the propagation of *S. pellita* by semihardwood stem cuttings, 2) investigate the rooting potential of single-node and leaf cuttings with and without exogenous auxin, and 3) compare rooting success across cuttings sourced from five geographically distinct *S. pellita* populations to identify potential population-level differences.

Materials and Methods

Substrate evaluation. Semihardwood cuttings were collected on 14 Aug 2024 from multiple stock plants of a single container-grown clone of *S. pellita* at the Plant Growth Facilities, University of Minnesota (UMN) Twin Cities St. Paul Campus, USA (lat. 44.988380 N, long. –93.181424W). These plants were originally sourced as dormant hardwood cuttings (dormant, lignified stems, comprising tissue from the previous growing season) from Vermont Willow Nursery (Fairfield, VT, USA) in May 2023, from a single genotype. Ten-centimeter cuttings were collected using a Felco 2 bypass pruner (Felco SA, Les Geneveys-sur-Coffrane, Val-de-Ruz, Switzerland), treated with 1000 ppm K-IBA (MilliporeSigma, Burlington, MA, USA) [dissolved in deionized (DI) water] via a 3-s quick dip of the basal third of the cutting, and inserted into individual cells of 50 cell trays (T.O. Plastics, Otsego, MN, USA; 27.9 × 53.9 × 5.7 cm). Cuttings were arranged in a completely randomized design (CRD) across each cell tray. Leaves were trimmed to 5 cm in length to reduce water loss during the experiment. Substrate treatments included 100% coarse perlite (Midwest Perlite, Appleton, WI, USA), 100% peat (Berger® BP Series Professional Sphagnum Peat Moss; Berger Peat Moss Ltd, Saint-Modeste, Quebec, Canada), and 50/50 v/v peat to perlite (N = 180 cuttings: 60 cuttings in 100% perlite, 60 cuttings in 100% peat, and 60 cuttings in 50/50 peat/perlite). Cuttings were placed in a mist bay greenhouse. Intermittent mist from a fine-mist nozzle positioned ~0.5 m above the bench was applied at 8-s intervals, every 4.5 min in Saint Paul, MN, USA. Every 15 min, photosynthetically active radiation (PAR) was recorded by an Apogee® SQ-500 Full-Spectrum Quantum Sensor (Apogee Instruments®, Inc., Logan, UT, USA). During this evaluation, the average PAR was 62.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with a maximum value of 819.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Greenhouse temperature and relative humidity were monitored by a HOBOconnect MX2302A (version 1.6.1; Onset Computer Corp; 470 MacArthur Blvd, Bourne, MA 02532, USA). The average daily temperature was 24.1 °C, with minimum night and maximum day values of 18 and 35.1 °C, respectively. Relative humidity averaged 74.8% and from 36.3% to 93.9%. Cuttings were evaluated 14 d posttreatment. Data collected were the total number of cuttings rooted per treatment, the number of roots per cutting, and the length of the five longest roots per cutting. Formation of callus and root initials were not measured. Rather, roots ≥ 0.25 cm in length were evaluated.

Cutting type and auxin treatment experiment. Single-node semihardwood stem cuttings and leaf cuttings were collected on 15 Aug 2025 from container-grown plants of the same *S. pellita* genotype used in the substrate evaluation. Samples were collected from multiple stock plants at the Plant Growth Facilities, UMN Twin Cities St. Paul Campus because of the number of propagules needed for this experiment. Single-node semihardwood (actively growing, semilignified tissue from the current growing season) stem cuttings (0.5-cm-long sections of stem with one node and leaf attached) as well as leaf cuttings (individual leaves trimmed from the stem at the proximal end of the petiole) were collected using a bypass pruner, and leaves were trimmed to 5 cm (removing distal lamina tissue) to reduce water loss of the propagules. The treatments in this experiment were single-node cuttings treated with 0 ppm (n = 50) and 1000 ppm (n = 50) K-IBA (dissolved in DI water) as well as leaf cuttings treated with 0 (n = 50) and 1000 (n = 50) ppm K-IBA. Propagules treated with auxin were treated via a 3-s quick dip of the basal third of the cutting. Cuttings (N = 200) were then inserted into 50-cell SunPack® Extra Strength Trays (Gempler's Inc., Janesville, WI, USA; 25.4 × 50.8 × 6.4 cm) filled with a 50/50 v/v mix of vermiculite (Horticultural Coarse Vermiculite; P.V.P. Industries Inc., North Bloomfield, OH, USA) and perlite (Midwest Perlite). Each experimental unit consisted of 10 subreplications arranged in two rows of five cuttings each. Each cutting was placed in an individual cell, with five cells per row within each tray. Cuttings were placed in a mist bay greenhouse using a CRD. Intermittent mist was applied for 8-s intervals every 4.5 min in Saint Paul, MN, USA. Every 15 min, PAR was recorded by an Apogee® SQ-500 Full-Spectrum Quantum Sensor (Apogee Instruments®, Inc). During this evaluation, the average PAR was 89 $\mu mol \cdot m^{-2} \cdot s^{-1}$, reaching a maximum value of 486.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Greenhouse temperature and relative humidity were monitored by a HOBOconnect MX2302A (v. 1.6.1; Onset Computer Corp). The average temperature was 23.3 °C, with minimum and maximum values of 16.2 and 35.1 °C, respectively. Relative humidity averaged 68.8% and ranged from 33.7% to 93.9%. Data were collected from cuttings 28 d after sticking. Data collected were the total number of cuttings rooted per treatment, the number of roots per cutting, and the length of the three longest roots per cutting. Roots ≥ 0.25 cm in length were evaluated. In addition, callus formation on leaf cuttings was recorded.

Population rooting trial. Semihardwood stem cuttings were collected on 18 Oct 2024 from four wild populations of *S. pellita*, including those found along the Cloquet River in MN, USA, and populations in Grand Portage, MN; Lutsen, MN; as well as Two Hearted, MI, USA. In addition, a greenhouse-grown genotype representing cultivated stock from Vermont Willow Nursery in Fairfield, VT, USA, was included. The sampled stems were taken from plants growing in a greenhouse on the St. Paul Campus of UMN. Stock plants were derived from wild populations sampled in Jun 2024 (week 2; from Two Hearted, MI, USA) and Jul 2024 (week 4; from Lutsen, the Cloquet River, and Grand Portage, MN, USA), then brought back to the Plant Growth Facilities on the St. Paul campus of UMN. Precise site location details are withheld in compliance with restrictions specified under the Minnesota Department of Natural Resources Special Permit No. 35134. Location data are presented at a spatial resolution no finer than an ~14.5-km radius, and site identifiers used adhere to these confidentiality requirements.

Ten-centimeter cuttings were collected using a bypass pruner, treated with 1000 ppm K-IBA (dissolved in DI water) via a 3-s quick dip of the basal third of the cutting, and subsequently inserted into individual cells of 50-cell trays (T.O. Plastics). To evaluate differences across populations, rooting success was analyzed with population as the independent variable, with n = 60 and N = 300. Distinct plants (genotypes) from wild collection sites were counted as separate individuals. Stem sampling occurred randomly across individuals representing each population. As in the substrate evaluation experiment, the leaves were trimmed to 5 cm to reduce water loss during root formation, and cuttings were placed in a mist bay greenhouse using a CRD. Every 15 min, PAR was recorded by an Apogee® SQ-500 Full-Spectrum Quantum Sensor (Apogee Instruments®, Inc). During this evaluation, the average PAR was 171.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, reaching a maximum value of 791.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Greenhouse temperature and relative humidity were monitored by a HOBOconnect MX2302A (version 1.6.1;

Onset Computer Corp). The average temperature was 22.2 °C, with minimum and maximum values of 16.4 and 28.8 °C, respectively. Relative humidity averaged 57.1% and ranged from 31.1% to 90.1%. Data were collected 14 d posttreatment with exogenous auxin. Data collected were the total number of cuttings rooted per treatment, the number of roots per cutting, and the length of the five longest roots per cutting. Roots ≥ 0.25 cm in length were evaluated.

Statistical analysis. One-to-one χ^2 tests were used to determine whether there was a significant association between categorical values [whether cuttings had rooted, using yes (+) or no (–) values] among overall rooting percentages for the substrate evaluation and the population rooting trial. One-way or two-way analyses of variance were used to determine whether there were significant differences among treatments for mean root length and mean root number. Data were analyzed using R Statistical Software (version 4.4.1; R Foundation For Statistical Computing, Vienna, Austria). The R package *tidyverse* was used to download and manage supporting packages, including *dplyr* for sorting and organizing data, and *ggplot2* for graphing mean root length and mean root number (Wickham 2016; Wickham et al. 2019, 2023). Mean separations among treatments were obtained using Tukey's honestly significant difference test at $\alpha = 0.05$ using the R package *multcompView* (Graves et al. 2019).

Results and Discussion

Substrate evaluation. According to the χ^2 analysis, overall rooting of semihardwood stem cuttings varied across treatments ($P < 0.001$), with 42%, 73%, and 100% rooting occurring with the 100% peat, 50/50 peat/perlite, and 100% perlite treatments, respectively. The mean number of roots was different across treatments ($P < 0.001$). Peat (100%) resulted in the lowest mean number of roots (Table 1). Compared with 100% peat, the number of roots increased by 263% and 1169% with the 50/50 mix of peat/perlite and 100% perlite, respectively (Table 1). Mean root length was also influenced by substrate type ($P < 0.001$). Peat (100%) and 50/50 peat/perlite yielded no differences and produced the shortest roots ($P = 0.976$), whereas 100% perlite resulted in the longest root lengths ($P < 0.001$) (Table 1).

Cutting type and auxin treatment experiment. Single-node cuttings rooted at 82% and 88% for the 0 and 1000 ppm exogenous auxin treatments, respectively. Leaf cuttings rooted at 66% and 32% for the leaf cuttings that did not receive exogenous auxin and the leaf cuttings that did receive exogenous auxin, respectively. For both mean root length and mean root number, the cutting type-by-auxin application interaction was not significant ($P = 0.128$ and $P = 0.718$, respectively). For mean root length, the main effect of cutting type was significant ($P < 0.001$), whereas auxin application was not ($P = 0.262$) (Table 2). Both single-node cutting treatments produced longer roots on average than both leaf-cutting treatments (Table 2). For mean root length, the single-node cutting treatments were not different from each other. For mean root number, auxin application had an effect ($P < 0.001$), whereas cutting type did not ($P = 0.187$) (Table 2). Treatments of exogenous auxin produced more roots on average compared than those without. Both single-node cutting treatments did exhibit propagules that produced shoots. No leaf cuttings produced shoots. However, 68% (68 of 100) produced callus.

Population rooting trial. Semihardwood stem cuttings rooted at 96.7% (58 of 60, Vermont Willow Nursery, Fairfield, VT, USA), 98.3% (59 of 60, Grand Portage, MN, USA), 98.3% (Cloquet River, MN, USA), 100% (Lutsen, MN, USA), and 100% (Two Hearted, MI, USA). With regard to overall rooting percentage, there was no difference among treatments according to the χ^2 analysis ($P = 0.2$). There was no difference in mean root length among treatments (Table 3). Thus, the pooled mean root length across treatments = 2.4 cm. The Grand Portage population produced the greatest number of roots on average and was different from the Cloquet River population ($P < 0.001$) (Table 3). In addition, the Vermont Willow Nursery and Grand Portage populations produced a greater number of roots on average and were different from the Cloquet River population ($P < 0.001$) (Table 3). Mean root number in all other populations was not different.

Yoon et al. (2021) investigated the effects of various rooting substrates and plant growth regulators on the propagation of *Salix koriyanagi* Kimura ex Goerz, a species distinct from *S. pellita*. Their study noted that phenolic foam, particularly when combined with 500 $mg \cdot L^{-1}$ IBA, yielded the greatest rooting percentage and strongest root development. Similarly, King et al. (2011) reported that substrates with enhanced aeration and drainage properties, such as perlite and phenolic foam, improved adventitious rooting consistently in baldcypress *Taxodium distichum* Rich. In alignment with these studies, our research focused specifically on *S. pellita* and evaluated substrates combined with K-IBA, finding that 100% perlite produced the greatest rooting percentage, as well as the greatest number and length of roots. Although a 50/50 peat/perlite mix resulted in a rooting rate of 73%, it produced significantly fewer and shorter roots. The use of 100% peat affected rooting negatively, likely because of poor aeration, causing cuttings to rot before rooting. These findings are supported by Milks et al. (1989), who noted hat the high air-filled porosity of perlite promotes root oxygenation and drainage, which are crucial factors influencing successful propagation outcomes. Yafuso et al. (2019) also emphasized the significance of aeration and drainage properties in propagation substrates, although perlite was not evaluated directly in their study. In addition, Ambebe et al. (2018) demonstrated that 100% sand, a substrate with excellent drainage and aeration, enhanced sprouting and shoot development significantly in evergreen tree species *Cordia africana* Lam. cuttings, whereas sawdust hindered rooting success because of the lack of drainage. Collectively, these studies emphasize that, despite species-specific variations in rooting response, substrates characterized by greater aeration and drainage enhance rooting success consistently across a range of diverse taxa.

Another factor influencing vegetative propagation success is cutting type (Hassanein 2013). Although leaf and single-node stem cuttings (∼0.5 cm) are not commonly used in *Salix* propagation, the threatened status of *S. pellita* and limited availability of plant material necessitate exploring efficient propagation methods. In our study, both cutting types treated with K-IBA exhibited more than 80% rooting success, indicating that single-node stem cuttings are a viable method for producing whole plants from limited material. Single-node cuttings have also been used successfully in the propagation of various woody species, with *Ficus carica* L. having been propagated from single-node cuttings with high success, particularly with 'Dottato', which demonstrated strong rooting and root development under controlled conditions (Mafrica et al. 2025). Together, these findings underscore the utility of alternative cutting types in rare plant conservation efforts where available material is limited. Although leaf cuttings did not form shoots, they did produce adventitious roots and callus.

In other species, leaf cuttings that produce callus have shown potential for whole-plant regeneration when treated with a cytokinin. For example, Cabahug et al. (2019) reported that kinetin stimulated shoot formation from callus tissue in *Echeveria* DC. species. However, results can be both species- and concentration-specific. For example, Jana et al. (2013) found that in *Sophora tonkinensis* Gagnep., 2-isopentenyladenine produced greater shoot multiplication than any tested concentration of

Table 1. Rooting responses (number of roots per stem cutting and root length) of semihardwood stem cuttings of *Salix pellita* 14 d after sticking in different soilless substrates.[i]

Treatment	No. of roots per stem	Mean root length (cm)
100% Peat	1.6 ± 0.3 c[ii]	0.5 ± 0.1 b
50/50 Peat/perlite	5.8 ± 0.7 b	0.6 ± 0.1 b
100% Perlite	20.3 ± 1.0 a	5.3 ± 0.3 a

[i] Substrates evaluated were 100% sphagnum peatmoss, 100% perlite, and a 50/50 peat/perlite mix. All cuttings were treated with 1000 ppm potassium-indole-3-butyric acid via a 3-s quick dip of the basal end of the cutting. Roots were counted if they were ≥ 0.25 cm.

[ii] Means with the same letter within a column are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Table 2. Rooting responses (no. of roots per cutting and root length) of single-node cuttings and leaf cuttings of *Salix pellita* 28 d after sticking.[i]

Cutting type	No. of roots per cutting	Mean root length (cm)
Leaf with auxin	5.2 ± 0.9 a[ii]	7.1 ± 0.9 b
Leaf without auxin	1.3 ± 0.4 b	4.2 ± 1.0 b
Node with auxin	5.7 ± 0.6 a	12.1 ± 0.7 a
Node without auxin	2.3 ± 0.2 b	12.1 ± 0.9 a

[i] Cuttings were stuck in a 50/50 perlite/vermiculite mix. Cuttings treated with auxin received 1000 ppm potassium-indole-3-butyric acid via a 3-s quick dip of the basal end of the cutting. Roots were counted if they were ≥ 0.25 cm.

[ii] Means with the same letter within a column are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Table 3. Rooting responses (no. of roots per stem cutting and root length) of semihardwood stem cuttings of *Salix pellita* from five distinct populations 14 d after sticking.[i]

Population[ii]	No. of roots per stem	Mean root length (cm)
MN-CR, St. Louis	10.7 ± 0.8 c[iii]	2.3 ± 0.1 a
MN-GP, Cook	17.7 ± 0.9 a	2.2 ± 0.1 a
MN-LT, Cook	13.3 ± 0.7 bc	2.3 ± 0.1 a
MI-TH, Luce	13.6 ± 0.6 bc	2.4 ± 0.2 a
VT-F, Franklin	16.0 ± 0.9 ab	2.7 ± 0.1 a

[i] All cuttings were treated with 1000 ppm potassium-indole-3-butyric acid via a 3-s quick dip of the basal end of the cutting. Roots were counted if they were ≥ 0.25 cm.

[ii] Population includes state–site and county, respectively, in the United States.

[iii] Means with the same letter within a column are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

MN-CR = Minnesota–Cloquet River; MN-GP = Minnesota–Grand Portage; MN-LT = Minnesota–Lutsen; MN-TH = Michigan–Two Hearted; VT-F = Vermont Willow Nursery.

kinetin or thidiazuron, while also promoting shoot elongation. These examples suggest that future research could explore the application of cytokinins to *S. pellita* leaf-cutting callus tissue to assess the potential for adventitious shoot development.

Previous research in *Salix psammophila* Wang & Yu, a shrub willow native to China, examined phenotypic variation in traits such as plant height, basal diameter, leaf area, and aboveground biomass among and within populations (Hao et al. 2019). That study found significant differentiation in these traits both within and among populations (Hao et al. 2019), suggesting underlying genetic diversity that may influence traits relevant to propagation success, including rooting capacity. The population-based rooting evaluation in our study showed no significant differences in overall rooting success between distinct wild and cultivated populations of *S. pellita*, indicating that propagation efforts need not be population specific. Broadly robust root numbers, averaging 10 or more roots per cutting across populations, indicate that population-level variation in this trait is unlikely to affect overall propagation success meaningfully.

Conclusion

The construction of an asexual propagation protocol for *S. pellita* will enable the establishment and management of a genetically diverse ex situ germplasm collection and strengthen conservation management strategies. Our study identified 100% perlite as the optimal rooting substrate, validated single-node cuttings as viable propagules for scenarios with limited plant material, and demonstrated that propagation outcomes are generally consistent across genetically distinct populations of the species.

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