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Engineered Sustainable Substitute: Roots of Seed-Propagated *Saposhnikovia divaricate* at Bolting-Stage as a Crop Engineering Solution for Wild Resource Replacement

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Abstract

Overharvesting of wild *Saposhnikovia divaricata*, the source of Saposhnikoviae Radix (SR), has posed critical ecological challenges and failed to meet industrial demand, necessitating engineered cultivation solutions. This study conducts an engineering assessment of roots from seed-propagated and root-cutting-propagated *S. divaricata* as technologically optimized substitutes for wild resources. Root samples from five major production regions were evaluated using both morphological characterization (in accordance with pharmacopoeia standards) and high-performance liquid chromatography (HPLC) for targeted phytochemical analysis of prim-*O*-glucosylcimifugin (POG) and 4'-*O*-*β*-D-glucosyl-5-O-methylvisamminol (GOM), collectively termed POGM (the sum of the POG and GOM contents). Engineered cultivation dynamics were further analyzed across four developmental stages (S1: rosette, S2: bolting, S3: immature fruit, and S4: fruit maturation) for seed-propagated *S. divaricata*. Results showed that roots from seed-propagated *S. divaricata* at the S2 exhibited morphological consistency with wild *S. divaricata* and comparable POGM levels (0.97% vs. 1.06%), meeting the engineered quality threshold (≥0.24%) specified by pharmacopoeia standards. In contrast, roots from root-cutting-propagated *S. divaricata* showed significant morphological deviations and sub-optimal POGM content (<0.70%). Stage-specific analysis revealed that S2-stage represent the optimal engineered harvest window, balancing root biomass accumulation and phytochemical expression. These findings validate an engineered cultivation strategy for SR, whereby seed-propagation combined with precise stage-based harvesting (S2) offers a scalable, eco-friendly technological solution to mitigate wild resource depletion. The study highlights the potential of crop engineering approaches in optimizing medicinal plant sustainability.

Keywords: Saposhnikovia divaricata; Seed-propagated; Bolting stage; wild vs cultivated medicinal resources.
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1. Introduction

Saposhnikoviae Radix (SR, Fangfeng) is a highly esteemed traditional Chinese medicine, widely recognized for its ability to dispel wind, alleviate pain, and relieve spasms. It plays a vital role in the East Asian herbal pharmacopeia, with a history spanning over two millennia.[1-4] The medicinal use of SR was recorded in Shen Nong's Materia Medica (Shen Nong Ben Cao Jing) as a premium-grade herb, indicating its high therapeutic value.[5] Traditionally, SR has been used to treat conditions such as colds, headaches, rheumatic arthralgia, rubella, itching, and tetanus due to its pungent, sweet, and slightly warming properties.[1] Over time, it has also become a popular herbal medicine in Japan and Korea, widely utilized for treating pain, inflammation, and arthritis.[6,7]

The source plant of SR, *Saposhnikovia divaricata* (Turcz.) Schischk, is a perennial herb in the Apiaceae family. It is distributed across Northeast Asia, which is characterized by a mid-temperate, semi-arid monsoon climate.[4,8] Despite its wide distribution, the wild resource of SR has faced increasing pressure due to continuous human exploitation. The supply-demand gap of SR in China, especially that derived from wild plants, was 200 tons in 2005 and increased to 3000 tons in 2013.[6] This growing demand has intensified pressure on wild populations, as unsustainable wild harvesting not only depletes natural reserves but also disrupts local ecosystems,

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such as soil erosion and loss of biodiversity in its native habitats.[8]

The sustainability of medicinal plants has garnered significant attention due to escalating demand and ecological degradation caused by wild harvesting. This necessitates a shift toward cultivation to ensure resource sustainability. However, traditional approaches to cultivation often fail to replicate the phytochemical profiles of wild plants, leading to quality inconsistencies that undermine clinical efficacy.[9] While cultivation alleviates ecological strain, it also presents challenges: domestication can alter the balance between growth and defense,[10] potentially accelerating growth rates, shortening vegetative phases, and modifying secondary metabolite accumulation compared with wild plants. For example, when the nutritional supply increases, carrots (*Daucus carota*) change from perennials to annual or biennials, and there is a phenomenon of accelerated flowering.[11] These potential phenotypic shifts raise concerns about quality consistency, as environmental standardization during domestication may inadvertently affect plant quality.[9] Investigating these changes is therefore essential to ensuring the medicinal integrity of SR.

SR's medicinal efficacy is strictly confined to the vegetative phase, with post-bolting roots losing therapeutic value. To ensure efficacy, both the Chinese Pharmacopoeia and Hong Kong Chinese Materia Medica Standard mandate SR harvesting before bolting.[1,12] However, artificial cultivation may alter phenological rhythms, potentially decoupling bolting from optimal metabolite synthesis. Current research lacks clarity on how cultivation-induced phenological affect pharmacologically active compounds, highlighting the need for refined quality control measures in cultivated SR.

The quality of traditional herbs is determined by morphological and chemical traits.[13] Morphologically, SR is characterized by a conical taproot, longitudinal wrinkles on the surface, and the presence of rootlet scars.[1,2,12,14] Chemically, SR differs from many Apiaceae plants, which primarily contain coumarins as their main bioactive components.[15-16] Instead, chromones are the crucial bioactive components in SR.[4,5,17,18] In particular, two chromones—4′-O-β-D-glucosyl-5-O-methylvisamminol (GOM) and prim-O-glucosylcimifugin (POG)— are the key bioactive constituents in SR. Collectively referred to as POGM, they exhibit anti-inflammatory, antioxidant, and analgesic activities.[19-21] The POGM is a certified quality indicator in the Chinese Pharmacopoeia and Hong Kong Chinese Materia Medica Standards. These standards mandate that POGM constitute at least 0.24% of the total composition.[1,12]

At present, there is a lack of comparative research on SR from engineered cultivated and wild plants, which cannot answer the question of whether SR from engineered cultivated plants can replace wild resources and how to replace them. Here, ‘engineered cultivation’ refers to systematic crop management approaches-including seed-propagation technology, stage-based harvesting protocols and environmental control-to mimic wild plant qualit under domesticated conditions. This strategy differs from genetic engineering, instead relying on agronomic optimization to balance biomass accumulation and secondary metabolite expression. This study proposed the hypothesis that SR from engineered cultivation at certain conditions can replace wild resources under certain conditions. To verify this hypothesis, this study compared the difference between engineered cultivation and wild SR and explored the influence of development on SR quality. Two engineered cultivation methods of SR, seed-propagation and root-cutting propagation, are included in the scope of this study.

This study aims to establish a crop-engineering-based approach for obtaining SR with optimal morphological and chemical properties. This is achieved by comparing wild and engineered cultivated SR from the key production regions and investigating roots of seed-propagated *S. divaricata* across developmental stages, with a focus on morphological traits and POGM content. The findings will provide novel insights into improving the medicinal quality of engineered-cultivated herbs and will support sustainable utilization.

2. Experimental

2.1 Sampling

Species identification was conducted by Dr. Chun-ying Ma at College of Agronomy, Hebei Agricultural University, China. Samples were collected from key production areas characterized by a mid-temperate, semi-arid monsoon climate in China (Fig. 1a, Table 1). Wild plants were collected from two locations: Zhalaiteqi county, Inner Mongolia (IMW, 46°43′N, 122°54′E), and Zhangbei Xiao’ertai Experimental Station, Zhangjiakou city, Hebei province (XEW, 41°11′N, 114°52′E). Engineered-cultivated samples included seed-propagated plants from Baoding city, Hebei province (BS, 38°48′N, 115°25′E), and root cutting-propagated plants from Anguo (AGR, 38°27′N, 115°16′E) and Quyang (QYR, 38°49′N, 114°33′E) counties, Hebei province. For comparison between wild and engineered-cultivated plants, all samples were harvested simultaneously in September 2020 at the S1 (rosette) stage to minimize potential confounding effects of seasonal or climatic variability on morphological and phytochemical comparisons (Table 1).

To evaluate developmental stage differences of seed-propagated plants, the samples were collected in September 2020 at four stages: S1 (rosette), S2 (bolting), S3 (immature fruit), and S4 (fruit maturation and senescence). After

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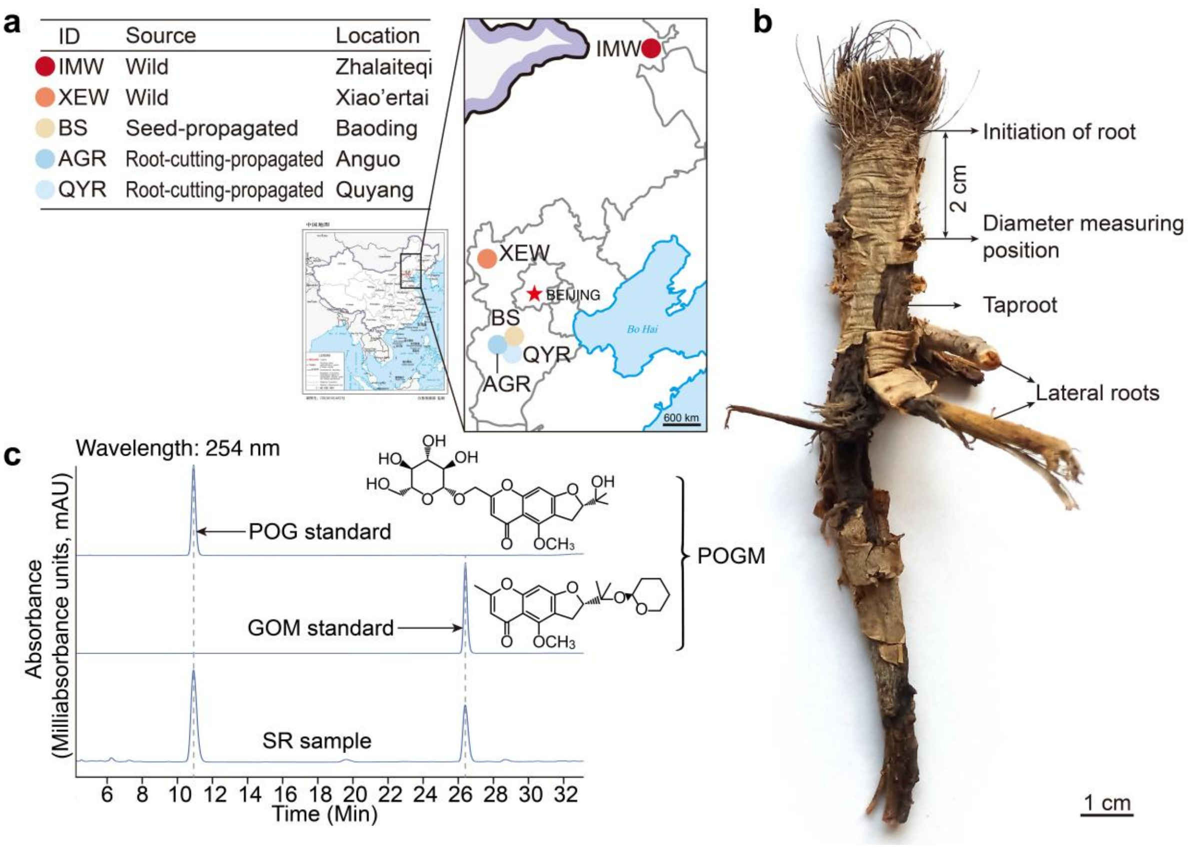

Fig. 1: Sampling locations, measurements and determination methods. (a) Sampling sites for this study. (b) Section and root diameter measuring site. (c) Representative chromatograms of standards and samples. From top to bottom, prim-*O*-glucosylcimifugin (POG) standard, 4'-*O*-*β*-D-glucosyl-5-O-methylvisamminol (GOM) standard, and Saposhnikoviae Radix (SR) sample.

Table 1: Sampling information for comparison of wild and engineered-cultivated plants.

ID	Source	Coordinates	Location
IMW	Wild	46°43′N, 122°54′E	Zhalaiteqi county, Inner Mongolia province, PRC
XEW	Wild	41°11′N, 114°52′E	Zhangbei Xiao'ertai Experimental Station, Zhangjiakou city, Hebei province, PRC
BS	Seed-propagated	38°48′N, 115°25′E	Experimental Station of Hebei Agricultural University, Baoding city, Hebei province, PRC
AGR	Root-cutting-propagated	38°27′N, 115°16′E	Anguo county, Hebei province, PRC
QYR	Root-cutting-propagated	38°49′N, 114°33′E	Quyang county, Hebei province, PRC

harvesting, the roots diameter and the roots weight were measured. Among them, the diameter of roots was measured at 2 cm below the root head (Fig. 1b) of *S. divaricata* using a vernier caliper, and the weight of roots was measured using an electronic scale. Three biological replicates were performed for each measurement. Subsequently, cross sections perpendicular to the vertical axis of the root 2 cm from the root head (Fig. 1b) were observed to assess the root cross section characteristics of the above four growth phases. The roots were subjected to HPLC analysis after being naturally dried.

2.2 Experimental design for engineered cultivation

The engineered cultivation experiments were conducted during 2019 and 2020 in Hebei province, China. For seed-propagation at the Experimental Station of Hebei Agricultural University, Baoding city, Hebei province, China. The seeds were sown in March 2019 across two experimental sites: an open plot measuring 666.7 m² and a 400 m² greenhouse. The row spacing was 25 cm with a seeding rate of 30 kg/hm^2, and a sowing depth of 1.5 cm. In 2020, field trials for root cutting propagation were conducted in two counties: Anguo and Quyang. The size for each experimental plot was 666.7 m² and the root cutting propagate methods were as follows: the roots derived from the 1-year-old plants were cut into 3-5 cm long segments, which were then planted in 3-5 cm depth in a population with row spacing of 30 cm and plant spacing of 15 cm. The experimental fields were fertilized before propagating. Weeds were removed before and during the seedling stage.

2.3 The POG and GOM content assay

2.3.1 Sample solution preparation and chromatographic conditions

Total contents of POG and GOM were determined according to the methods described in the Chinese Pharmacopoeia.[1] The dried roots were ground into powder and passed through a sieve (40 mesh). The constituents of the root powder were extracted from a 0.25 g sample in 10 mL of methanol by reflux in a water bath for two hours at 72°C, followed by filtration through a membrane after cooling to room temperature. The resulting filtrate from the sample extract solution was used for HPLC analysis under the following conditions using the Agilent 1260 Infinity II System (Agilent Technologies, Inc., Santa Clara, CA, USA): ZORBAX Eclipse Plus C18 (4.6 × 250 mm, 5 μm; Agilent Technologies, Inc.) column; mobile phase A, purified water; mobile phase B, methanol; flow rate, 1.0 mL/min; detection wavelength, 254 nm; injection volume 2 μL; column temperature, 35°C. The gradient elution procedure for HPLC is shown in Table 2. The contents of POG and GOM were calculated using the external standard method. Fig. 1c shows representative chromatograms of POG and GOM standards and a sample.

Table 2: HPLC gradient elution procedure.

Time (min)	A (%)	B (%)	Flowing (mL/min)
0-15	65	35	1
15-35	65-45	35-55	1

2.3.2 The POG and GOM reference solution preparation and standard curve generation

The POG and GOM reference substances (Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China) were used to establish a standard curve. Table 3 shows the regression equations and linear range of standard curves for POG and GOM.

To obtain the POG standard stock solution (1.64 mg/mL), POG standard (16.4 mg) was dissolved in 10 mL of chromatographic methanol. A concentration gradient of POG standard solution was established by diluting stock solution (0.5 mL) with various amounts of methanol (40.5, 13.0, 7.5, 5.0, 4.0 mL) for later use. Likewise, GOM standard (21.4 mg) was dissolved in 10 mL of chromatographic methanol to obtain a standard stock solution (2.14 mg/mL). The GOM stock solution (0.5 mL) was then used to construct a concentration gradient of GOM standard solution after dilution with methanol (53.0, 17.0, 10.0, 7.0, 6.5 mL) for later use.

The reference solutions were analyzed under the chromatographic conditions described in 2.3.1, with an injection volume of 2 μL. A standard curve was plotted with the mass (μg) as the abscissa and the peak area as the ordinate. The results indicated that linear relationships for POG and GOM were observed within the ranges of 0.0400–0.3644 μg and 0.0400–0.3567 μg, respectively (Table 3).

2.4 Data processing and analysis

The measured indices of all samples in this experiment were performed in three replicates. Excel 2010 (Microsoft Corp., Redmond, WA, USA) was used to calculate the average fresh weight and diameter of the roots of *S. divaricata.* The contents of POG and GOM were calculated based on the corresponding standard curves using Excel 2010. The POGM contents of the samples were the sum of the POG and GOM contents. The POGM content of each root was obtained by multiplying the weight of each root by the percentage of POGM. One-way ANOVA analysis of variance with SPSS statistics 26.0 (IBM Corp., Armonk, NY, USA) was performed to identify statistically significant effects of the sample source on agronomic traits and contents of POG, GOM, POGM. The LSD was used to compare sample means while controlling multiple comparisons. In all tables and figures, different lowercase letters denoted significant differences ($P < 0.05$).

3. Results and discussion

This study comprehensively evaluated the morphological traits and POGM content of *S. divaricata* roots from both wild and engineered cultivated sources in major production regions, aiming to assess the suitability of engineered cultivated SR as an alternative for wild SR.

3.1 Morphological comparison between SR from the wild and engineered cultivation

All sampling sites are representative production zones of SR, including two wild sites (IMW and XEW) and three engineered cultivation sites (BS, AGR, and QYR) (Fig. 1a). The plant morphology is consistent with the records of Flora of Chinese, which supports the correctness of this species (http://www.iplant.cn). Wild and engineered cultivated plants exhibited distinct phenological phases. Wild *S. divaricata* plants are perennials with uncertain growth durations. Among

Table 3: Properties of standard curves.

Standard ^{a}	Regression equation	r^2	Linear range (μg)
POG	y=1.2180 + 1916.1552x	0.9998	0.0400 – 0.3644
GOM	y=-12.4561 + 1752.9776x	0.9996	0.0400 – 0.3567

^{a} POG: prim-*O*-glucosylcimifugin; GOM: 4’-*O*-*β*-D-glucosyl-5-O-methylvisamminol.

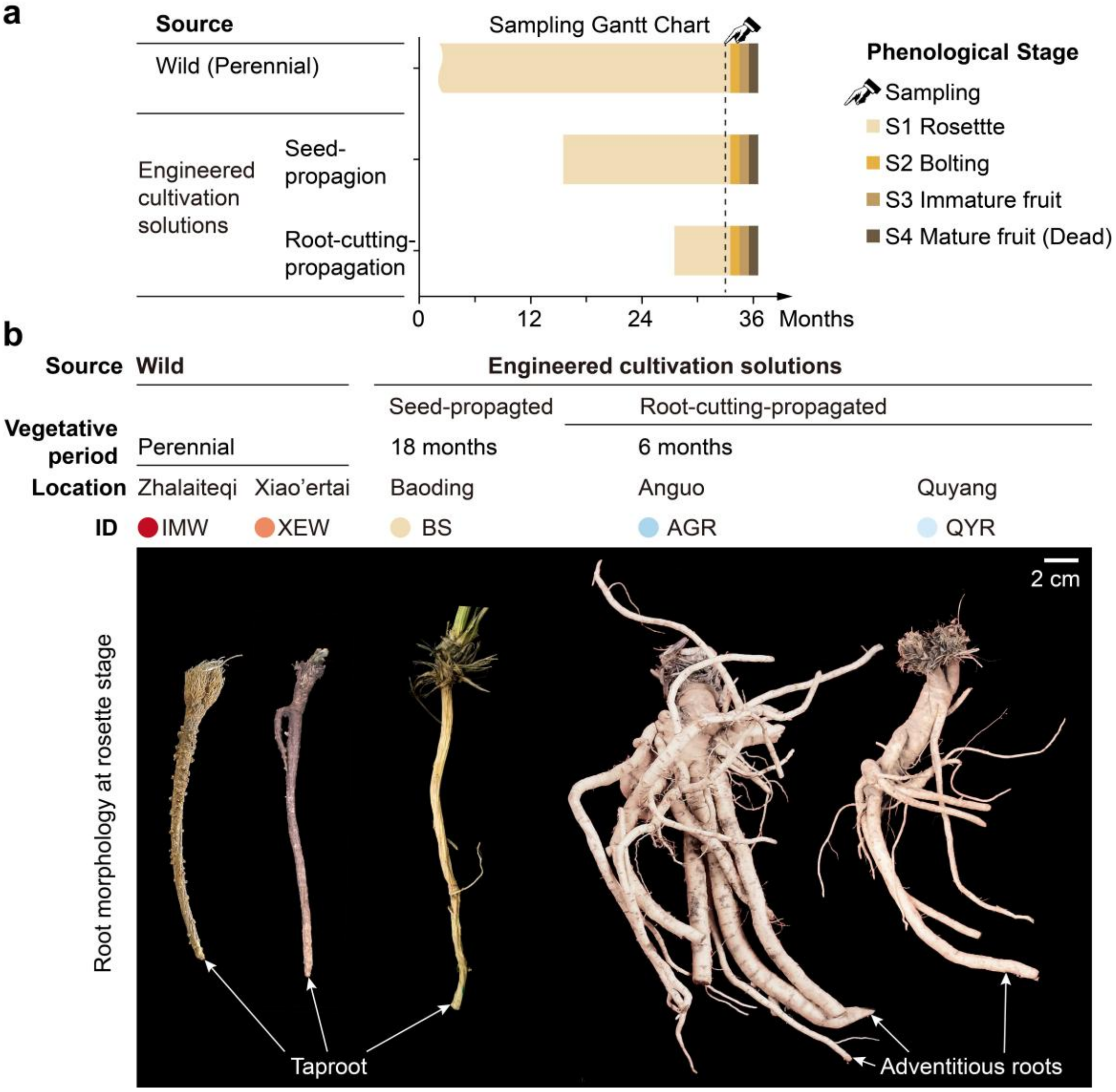

Fig. 2: Morphological comparison of *S. divaricata* roots from different sources. (a) Gantt chart illustrating the sampling timeline for specimens from wild populations, seed-propagated plants, and root cutting-propagated plants, with meticulously designed breeding and sampling schedules to ensure consistency and minimize seasonal or climatic influences. (b) Photographic representation of *S. divaricata* roots from three sources (wild, seed-propagation, and root-cutting propagation) and five geographical locations, demonstrating that seed-propagated roots, characterized by a prominent taproot, closely resemble wild samples, while root-cutting-propagated roots exhibit numerous well-developed adventitious roots.

engineered cultivated plants, seed-propagated samples exhibit a biennial growth cycle, being sown in March of the first year, bolting after 18 months, dying in the second winter. In contrast, root-cutting-propagated *S. divaricata* follow an annual cycle, as they are planted in March, completed the vegetative period within six months, bolt, and die in the first winter (Fig. 2a).

The results show that under artificial environment, the plants change from wild perennials to annual or biennials. It may be that the artificial environment has sufficient nutrients, which accelerates the flowering of the plants. This phenomenon also occurs in carrots,[10] a plant of the same Apiaceae family. The transition from perennial wild *S. divaricata* plants to annual or biennial cultivated *S. divaricata* plants may reflect changes in stress-resistance and growth strategies induced by cultivation practices.[9]

To minimize the impact of seasonal and climate variability, samples were harvested simultaneously, yet wild and cultivated SR exhibited distinct phenotypes (Fig. 2). The morphological traits of SR from wild and engineered cultivated *S. divaricata* were examined (Fig. 2b, Table 4). The wild IMW sample displayed morphology most closely aligned with the Chinese Pharmacopoeia standards, including the lenticel-like protrusions and punctate fine root marks. The XEW sample closely resembled IMW, with similar taproot shape, color, but no lenticel-like protrusions and punctate fine root marks. In contrast, SR samples from engineered cultivation exhibited significant differences: SR from seed-propagation *S. divaricata* (BS) resembled wild SR with a distinct taproot, whereas SR samples from root-cutting-propagated *S. divaricata* (AGR and QYR) lacked a clear taproot and had numerous developed adventitious roots, deviating from both wild SR and pharmacopoeia standards.

Besides, the SR from the wild were darker in color than those from engineered cultivation.

Adherence to pharmacopoeia standards is crucial for ensuring the quality and safety of medicinal materials.[22] Both appearance and chemical composition are key indices for assessing quality.[13,23] According to the Chinese Pharmacopoeia and Hong Kong Chinese Materia Medica Standards, only pre-bolted *S. divaricata* roots meet the required phenotypic and chemical standards.[1,12] These standards specify long, conical or cylindrical roots with tapered, occasionally curved ends, grayish-brown to tan surfaces, longitudinal wrinkles, lenticel-like protrusions, and punctate fine root scars.[1,2,12,14] The results indicate that both wild SR and SR from seed-propagated *S. divaricata* closely align with key pharmacopoeia morphological criteria, particularly the presence of a conical taproot. However, the absence of lenticel-like protrusions in wild SR samples (XEW) and SR from seed-propagated *S. divaricata* (BS) suggests that even wild populations do not always fully conform to pharmacopoeia descriptions. In contrast, SR from root-cutting-propagated *S. divaricata* completely deviated from these standards due to its asexual propagation from root segments, resulting in the absence of a primary taproot and the development of numerous adventitious roots. This structural difference likely contributed to the lower quality of SR from root-cutting-propagated *S. divaricata*.

Historically, adventitious roots have been considered detrimental to SR quality and were traditionally removed to enhance medicinal value. However, appearance-based quality evaluation is rooted in the experience of wild-harvested herbs, which may not be entirely applicable to cultivated varieties. Engineered cultivation alters environmental conditions, potentially shifting the balance between growth and defense strategies in plants.[10] Further research is needed to clarify which morphological traits are truly critical for SR quality and which are less relevant, ensuring that evaluation standards reflect both traditional knowledge and modern cultivation practices.[13]

3.2 Comparison of POGM content between SR from the wild and engineered cultivation

Root POGM levels in both wild and engineered cultivated *S. divaricata* samples ranged from 0.40% to 1.09%, all of which were higher than the minimum standard (≥0.24%) specified by the Chinese Pharmacopoeia and Hong Kong Chinese Materia Medica Standard. Among wild samples, IMW and XEW exhibited high POGM contents (>1.00%), with IMW samples fully conforming to the morphological standards of the national Pharmacopoeias.[1,2,12,14] Interestingly, despite lacking lenticel-like protrusions and fine root scars, XEW (1.09%) had a higher POGM content than IMW (1.03%), suggesting that some external morphological features may not reliably predict active components concentrations. Considering that the quality evaluation of traditional Chinese medicine emphasizes the integration of morphology and effect,[13] these findings highlight the need for further investigation into morphological and physiological factors that strongly correlate with SR quality and pharmacological efficacy.

The POGM contents in engineered cultivated SR (<0.70%) were consistently lower than those in wild SR (>1.00%) (Table 5). The disparity may be attributed to differences in defense-growth trade-offs shaped by natural versus artificial environments.[10,24] Additionally, the higher POGM content in wild samples likely results from their perennial growth habit and extended vegetative period, which facilitate greater metabolite accumulation.[9]

Given that rosette stage (S1) roots of seed-propagated *S. divaricata* (BS) showed a POGM content of 0.48% significantly lower than wild samples (1.03-1.09%, Table 5) and that root-cutting-propagated plants exhibited both morphological deviations (*e.g.*, absence of a distinct taproot, Table 4, Fig. 2b) and suboptimal POGM levels (<0.70%), we hypothesized that both morphological attributes and POGM content vary dynamically across developmental stages ofseed-propagated *S. divaricata*. This hypothesis was further informed by the oberservation that morphological deviations in root-cutting-propagation such as the development of

Table 4: Growth Habit and root morphology of *S. divaricata* from different sources.

Source	ID ^{x}	Growth Habit	Morphology			
			Distinct taproot	Rough epidermal with longitudinal wrinkles	Lenticular protuberances and punctate root scars	Color
Wild	IMW	Perennial	Yes	Yes	Yes	Dark
Wild	XEW	Perennial	Yes	Yes	No	Dark
Seed-propagated	BS	Biennial	Yes	Yes	No	Taupe
Root-cutting-propagated	AGR	Annual	No	Yes	No	Pale
Root-cutting-propagated	QYR	Annual	No	Yes	No	Pale

^{x} IMW: Wild sample in Zhalaiteqi county, Inner Mongolia; XEW: Wild sample in Zhangjiakou city, Hebei province; BS: Seed-propagated sample in Baoding city, Hebei province; AGR: Root-cutting-propagated sample in Anguo county, Hebei province; QYR: Root-cutting-propagated sample in Quyang county, Hebei province.

Table 5: POGM level of *S. divaricata* from different sources.

Source	ID ^{x}	POG ^{y} (%)	GOM (%)	POGM (%)
Wild	IMW	0.62 ± 0.009^a	0.41 ± 0.004^b	1.03 ± 0.010^b
Wild	XEW	0.64 ± 0.014^a	0.45 ± 0.007^a	1.09 ± 0.014^a
Seed-propagated	BS	0.35 ± 0.017^c	0.14 ± 0.007^e	0.48 ± 0.024^d
Root-cuttingpropagated	AGR	0.45 ± 0.004^b	0.16 ± 0.003^d	0.62 ± 0.002^c
Root-cutting-propagated	QYR	0.21 ± 0.001^d	0.18 ± 0.002^c	0.40 ± 0.002^e

^{x} IMW: Wild sample in Zhalaiteqi county, Inner Mongolia; XEW: Wild sample in Zhangjiakou city, Hebei province; BS: Seed-propagated sample in Baoding city, Hebei province; AGR: Root-cutting-propagated sample in Anguo county, Hebei province; QYR: Root-cutting-propagated sample in Quyang county, Hebei province.

^{y} POG: prim-*O*-glucosylcimifugin; GOM: 4'-*O*-*β*-D-glucosyl-5-O-methylvisamminol. Values are presented as mean ± standard deviation (SD); Different letters at each column denote significant differences in Duncan's ANOVA comparison ($P < 0.05$).

adventitious roots — correlated with reduced POGM accumulation, implying a potential link between root structure and secondary metabolism.

Guided by this hypothesis, we investigated the morphological and chemical dynamics of roots from seed-propagated *S. divaricata* across four developmental stages (S1-S4). These stages (S2-S4) were explored whether the observed POGM deficit in S1-stage cultivated roots could be mitigated by targeting a later developmental window, thereby bridging the quality gap between wild and engineered cultivated SR.

3.3 Morphological comparison of SR from seed-propagated plants at different development stages

The four time points selected in this study represent key developmental stages of *S. divaricata*, each marked by significant morphological changes (Fig. 3). In the rosette stage (S1), the plant develops green leaves, followed by bolting (S2), immature fruits formation (S3), and finally, mature fruits and plant senescence(S4). During S1, roots are fragile and prone to breakage. In contrast, from S2 to S3, root surface becomes brownish-yellow, dry, and structurally more robust. By S4, the roots are darkened, desiccated, and structurally compromised, with reduced phloem proportion and distinct separation of phloem and xylem.

Cross-sectional observations further revealed developmental changes in root structure (Fig.3a). At S1, the phloem was thick and white, whereas the xylem was pale yellow. As plants transitioned to S2 and S3, phloem thickness decreased, and xylem darkened, with cracks appearing in the bolting stage and intensifying in the immature fruit stage. From S3, the wrinkles appeared obviously, and the roots became tough in structure, which may represent the loss of various active ingredients and nutrients. By S4, the boundaries between phloem and xylem became indistinct, and root tissue exhibited significant structural degradation, ultimately leading to plant death (Fig. 3a). While *S. divaricata* is botanically classified as a perennial herb (http://www.iplant.cn), this study reveals that following flowering and seed set, its root undergoes rapid lignification and structural degradation, resulting in hollowing and a complete loss of morphological compliance with Pharmacopoeia standards. This irreversible shift from vegetative to reproductive growth hence aligns with the functional definition of a monocarpic plant, which flowers only once before senescence. This is also an important finding of this study.

Developmental stages: S1 (rosette), S2 (bolting), S3 (immature fruit), and S4 (fruit maturation and senescence). BS1-BS4 and GS1-GS4 represent seed-propagated plants at the S1-S4 stages grown in an open field (BS) and a greenhouse (GS) in Baoding, respectively.

3.4 POGM dynamics and optimal harvesting stage

Agronomic traits and POGM content varied significantly across developmental stages (Fig. 3b-3h and Table 6). Root diameter, weight, and POGM content increased during growth, peaking at S2 before declining. The highest POGM content (0.9728%) was recorded at S2 (Fig. 3h and Table 6). Greenhouse experiments conducted alongside field trials confirmed this trend (Fig. 3f-3h and Table 7), supporting S2 as the optimal harvesting period. This represents a pivotal finding: the bolting stage, when properly engineered, balances root development and secondary metabolism, offering a sustainable alternative to wild SR. However, the difference in POGM content at four developmental stages between field-grown and greenhouse indicates that environmental factors such as temperature and light intensity may play a role. This will be a key area of focus in our future research to further clarify their specific impacts on POGM synthesis and accumulation.

As a monocarpic plant, the *S. divaricata* senesces after bolting and dies upon fruit maturation.[25] Monocarpic senescence phenomenon is not uncommon among the Umbelliferae family plants, such as carrots.[11] The decline in POGM post-bolting aligns with previous research. From a pharmacological perspective, polysaccharide degradation after bolting has been suggested as a primary factor in the reduction of SR efficacy.[26] This study proposes new insights from the perspective of plant growth and development, that the primary cause of diminished efficacy post-bolting is likely

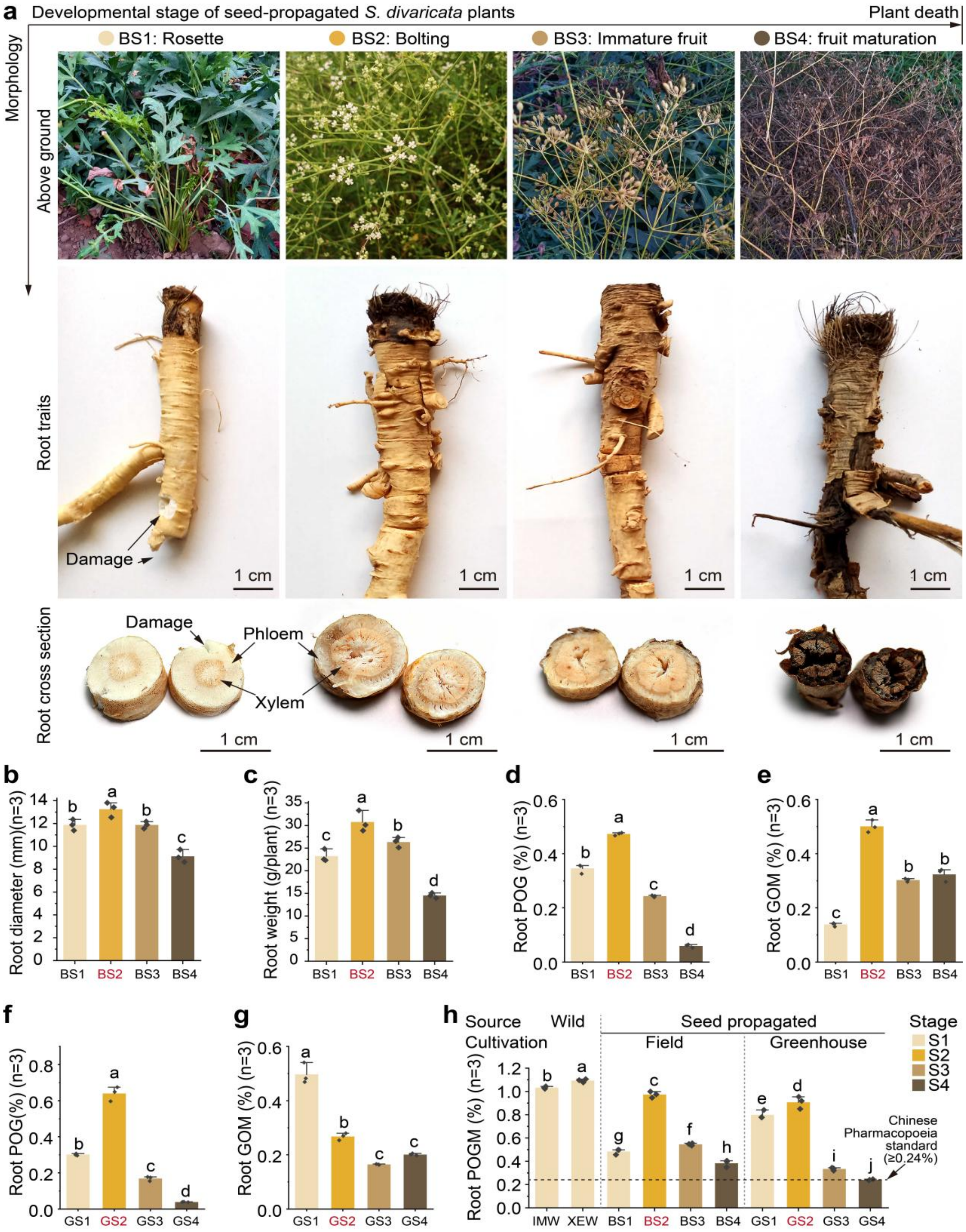

Fig. 3: Morphological traits and root POGM levels of seed-propagated *S. divaricata* across distinct developmental stages.(a) Representative images of plant morphology, roots, and root cross-sections. Quantitative analyses of root (b) diameter, (c) weight, and (d) POGM levels. Results demonstrate that roots from seed-propagated *S. divaricata* at the S2 stage most closely resemble the quality of wild medicinal materials.

due to an explosion of monocarpic senescence,[25] including polysaccharide and POGM decline. The present study provides new evidence that POGM levels drop significantly post bolting, falling to less than half of their peak value at S2. This suggests that rather than polysaccharide loss alone, the sharp decline in overall metabolic activity due to monocarpic senescence may be the primary factor driving reduced SR efficacy after bolting.

Table 6: POGM levels of roots from seed-propagated *S. divaricata* at test field.

Stage	Label	POG ^{x} (%)	GOM (%)	POGM (%)
S1	BS1	0.3453±0.017^b	0.1387±0.007^c	0.4839±0.024^c
S2	BS2	0.4723±0.005^a	0.5005±0.023^a	0.9728±0.026^a
S3	BS3	0.2430±0.004^c	0.3025±0.006^b	0.5455±0.010^b
S4	BS4	0.0595±0.006^d	0.3233±0.024^b	0.3828±0.031^d

^{x} Values are presented as mean ±SD; Different letters at each column denote significant differences in Duncan's ANOVA comparison ($P < 0.05$).

Table 7: POGM levels of roots from seed-propagated *S. divaricata* in greenhouses.

Stage	Label	POG ^{x} (%)	GOM (%)	POGM (%)
S1	GS1	0.3014±0.006^b	0.4973±0.038^a	0.7986±0.037^b
S2	GS2	0.6394±0.040^a	0.2676±0.013^b	0.9071±0.053^a
S3	GS3	0.1691±0.013^c	0.1647±0.001^c	0.3338±0.013^c
S4	GS4	0.0391±0.001^d	0.2012±0.004^c	0.2403±0.005^d

^{x} Values are presented as mean ± SD; Different letters at each column denote significant differences in Duncan's ANOVA comparison ($P < 0.05$).

Given that POGM levels peak at S2 and decline thereafter, harvesting at this stage maximizes both yield and medicinal quality. Historically, wild SR harvesting was imprecise due to environmental variability, making it difficult to ensure optimal POGM content. However, controlled artificial cultivation offers the opportunity to precisely time harvests at peak POGM levels. Integrating the findings of Yang et al. (2016) with this study, harvesting before senescence—when POGM is high, and polysaccharides remain abundant—ensures a pharmacologically superior product.

The discovery that *S. divaricata* is a monocarpic plant, combined with the finding of high POGM content at the bolting stage (S2), paves the way for engineered cultivation strategies: Phenological regulation to extend the vegetative phase via environmental control for enhanced POGM accumulation. Stage-specific harvesting at S2, aligning with the peak of secondary metabolism before irreversible lignification. These insights integrate botanical phenology with crop engineering to bridge wild resource gaps sustainably.

3.5 Implications for pharmacopoeia standards

Currently, the Chinese Pharmacopoeia and Hong Kong Chinese Materia Medica Standard specify that SR should be harvested before bolting.[1,12] However, results from this study suggest that the bolting stage (S2) is superior to the currently recommended rosette stage (S1), as it yields higher POGM content while maintaining structural integrity and meeting pharmacopoeia standards.

The peak POGM levels at S2 likely result from intensified secondary metabolism during early reproductive growth, a pattern observed in other medicinal plants such as *Panax ginseng* C A Meyer[27] and *Callerya speciosa* (Champ. ex Benth.) Schot,[28] where active compound accumulation increases with plant maturity. Notably, at S2, the POGM content is twice that of S1, marking a critical phase where biomass accumulation surpasses nutrient consumption, further enhancing medicinal quality. This conclusion is reinforced by consistent findings across both field and greenhouse experiments.

Given that POGM levels decline post-bolting, extending the vegetative phase may provide a strategy to further optimize POGM accumulation. The WRKY1 gene, known to regulate monocarpic senescence in *Arabidopsis thaliana* (L.) Heynh.,[29] could play a role in prolonging vegetative growth in *S. divaricata*, warranting further investigation into its potential application in optimizing SR cultivation.

3.6 Comparison of POGM content in SR from the wild and seed-propagated plants across growth phases

The root POGM content of seed-propagated *S. divaricata* was significantly lower than that of wild plants (1.06% at S1 stage) across all growth phases (Fig. 3d). Specifically, POGM levels in roots from seed-propagated *S. divaricata* were reduced by approximately 50% at S1 (0.4839%), S3 (0.5455%), and S4 (0.3828%) stages. However, roots harvested at the S2 stage (0.9728%) exhibited a much smaller reduction in POGM content, with levels approaching those of wild plants, highlighting the potential of SR at S2-stage seed-propagated *S. divaricata* as a viable alternative with relatively higher medicinal quality. Notably, S2 roots also exhibited superior agronomic traits, including the highest weight and diameter among all growth phases (Fig. 3b–3d, Tables 5–7). These findings suggest that seed-propagated *S. divaricata* harvested at the S2 stage represents a promising alternative to wild plants, combining favorable morphological characteristics and medicinal quality (POGM content) for potential use in SR production. This genetic diversity discrepancy may directly influence the biosynthesis and accumulation of active components such as POGM, thereby explaining the differences in medicinal quality between wild and cultivated *S. divaricata*. For example, Wang et al. have pointed out that wild plant populations generally exhibit higher genetic diversity, while cultivated plant populations tend to have more conservative genetic diversity after domestication, which may result in lower contents of certain medicinal components in cultivated medicinal materials compared to wild ones.[30]

Overall, the findings of this study suggest that seed-propagated *S. divaricata* harvested at the S2 stage can serve as a viable and sustainable alternative to wild SR. However, the results differ from prior studies, the Chinese Pharmacopoeia, and the Hong Kong Chinese Materia Medica Standard, which discourage the use of bolting plants for SR production.[1,12,30] This discrepancy underscores the need for further research to explore chromone accumulation dynamics and validate the suitability of bolting plants for SR production. Implementing the S2-stage, seed-propagation system would decouple SR production from wild collection, supporting national conservation goals while ensuring consistent raw-material quality for the herbal-medicine industry.

3.7. Considerations for regional adaptation and scalability

While our study demonstrates the viability of the "seed-propagation + S2 harvesting" strategy in Hebei Province, its broader application requires addressing climatic variability (*e.g.*, differences between Hebei and Inner Mongolia). Future multi-regional trials across Northeast, Northwest, and East China will systematically evaluate how temperature, precipitation, and photoperiod influence bolting phenology and POGM accumulation, enabling region-specific harvest timing optimization. Furthermore, scalability hinges on integrating agronomic and economic factors (*e.g.*, cultivation costs, labor efficiency, and market demand), which will be quantified in subsequent studies to ensure practical feasibility.

4. Conclusion

Seed-propagated *S. divaricata* harvested at the bolting stage (S2) produces roots that equal wild-harvested SR in key morphology and POGM content (~0.97 % vs 1.06 %). These roots satisfy pharmacopoeial standards. Consequently, seed-propagation combined with S2-stage harvest provides a scalable, sustainable replacement for wild SR. Further study should focus on (i) validate this protocol in multi-regional field trials, (ii) refine agronomic parameters for consistent POGM yields, (iii) conduct economic analyses of large-scale implementation, and (iv) investigate genetic approaches to widen the optimal harvest window.

Declaration of AI technologies in the writing process

The authors utilized ChatGPT and DeepSeek for language refinement during manuscript preparation. All content was rigorously reviewed and revised by the authors, who take full responsibility for the final version.

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Conflict of Interest

There is no conflict of interest.

CRediT Statement

Zhang Qingqing: Investigation, Data Curation, Writing-Original Draft; **Wu Lizhu:** Methodology, Data Curation; **Chen Xiaolu:** Formal analysis, Visualization, Writing-Reviewing and Editing; **Hu Yanguang:** Methodology, Project administration; **Tao Mingshuang:** Resources, Validation; **Wang Jing:** Investigation, Formal analysis; **Ma Chunying:** Funding acquisition, Supervision, Writing-Reviewing and Editing.

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