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Research Insight

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Analysis of the Mechanisms by Which Shading Environment Affects the Growth and Medicinal Quality Formation of *Tetrastigma hemsleyanum*

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Abstract This study explores the mechanisms by which shading environments affect the growth and medicinal quality formation of *Tetrastigma hemsleyanum*. *T. hemsleyanum* is an important medicinal vine in China, and its tuberous roots and aerial parts are rich in bioactive compounds, including flavonoids, polysaccharides, and phenolic acids, which have medicinal development value due to their anti-inflammatory, antioxidant, immunomodulatory, and antitumor activities. With the decline of wild resources and the increasing demand for artificial cultivation, achieving high-yield and high-quality production of *T. hemsleyanum* through light environment regulation has become a key issue in its standardized cultivation and industrial development. This study analyzes the ecological habits and low-light adaptation basis of *T. hemsleyanum*, elucidates the effects of shading on morphogenesis, biomass accumulation, photosynthetic pigments, photosynthetic efficiency, antioxidant systems, carbon-nitrogen metabolic balance, and the accumulation of flavonoids, polysaccharides, and phenolic compounds, and further discusses the roles of light signal perception, signal transduction pathways such as COP1-HY5, key enzyme genes, and transcription factor regulation in medicinal quality formation. Existing studies indicate that *T. hemsleyanum* exhibits obvious shade-adaptive characteristics, and approximately 67%~70% shading is generally beneficial for maintaining relatively high net photosynthetic rate, stomatal conductance, and leaf function, while also promoting vine growth, leaf expansion, and tuberous root development. In contrast, strong light or excessive shading may inhibit photosynthetic efficiency and disrupt carbon assimilation and biomass allocation. In the future, shading intensity, light quality regulation, cultivation model optimization, and multi-index quality evaluation systems should be integrated to establish standardized shading cultivation techniques suitable for different ecological regions and growth stages of *T. hemsleyanum*, thereby providing a theoretical basis for resource conservation, high-quality medicinal material production, and industrial utilization.

Keywords *Tetrastigma hemsleyanum*; shading environment; photosynthetic physiology; secondary metabolism; medicinal quality

1 Introduction

Tetrastigma hemsleyanum is a high-value Chinese medicinal vine, and both its roots and aerial parts are widely used. With increasing market demand and intensified overharvesting, the utilization of *T. hemsleyanum* resources is rapidly shifting from wild collection to artificial cultivation (Hu et al., 2021). Its medicinal quality largely depends on light-sensitive secondary metabolites; therefore, shading management has become a key issue in standardized and high-quality production (Zhang et al., 2021). Traditionally, *T. hemsleyanum* has been used to treat fever, pneumonia, asthma, hepatitis, rheumatism, and other inflammatory and infectious diseases, and it is known in folk practice as a “natural plant antibiotic” (Ji et al., 2020). Modern studies have identified more than 140 compounds in this species, among which flavonoids and polysaccharides are representative active components, exhibiting antitumor, anti-inflammatory, antioxidant, immunomodulatory, and antipyretic activities. Total flavonoids from the roots of *T. hemsleyanum* can inhibit colorectal tumor growth and regulate gut microbiota (Han et al., 2023), while polysaccharides from the roots and aerial parts show significant antitumor, antipyretic, and immunomodulatory effects in vivo. These findings indicate that *T. hemsleyanum* is a potential resource for developing novel antitumor and immunomodulatory products and also provide strong impetus for its industrial development.

For medicinal plants, light environment regulation is a core agronomic measure that simultaneously affects biomass and secondary metabolite accumulation (Zhang et al., 2021). In many medicinal plants, shading or light

intensity regulation has been shown to alter photosynthesis, pigment content, and the levels of phenolics, flavonoids, and other bioactive compounds. The optimal state often occurs under moderate shading rather than full sunlight or deep shading (Xu et al., 2020; Gao et al., 2025). In shade-tolerant or understory herbaceous medicinal plants, appropriate shading can simultaneously increase aboveground yield and enhance the accumulation of specific medicinal metabolites, thereby improving overall medicinal value. However, plant responses to the light environment are highly species-specific and are jointly affected by light intensity, spectral quality, and duration. Therefore, it is difficult to directly apply a universal “light formula” to all medicinal crops.

This study explores the mechanisms by which shading environments affect the growth and medicinal quality formation of *T. hemsleyanum*. Existing studies on the physiological characteristics of *T. hemsleyanum* have only briefly addressed light effects, and there remains a clear knowledge gap regarding how the light environment shapes its growth process and medicinal quality formation. Given the increasing scarcity of wild resources and the rapid expansion of cultivated areas, it is necessary to clarify the mechanisms by which shading environments regulate the growth of *T. hemsleyanum* and the accumulation of key active components. On the one hand, standardized light and shading management is important for improving biomass, stabilizing quality markers such as flavonoids and polysaccharides, supporting quality control, and promoting industrial upgrading. On the other hand, clarifying how light intensity and shading affect photosynthesis, antioxidant defense, and secondary metabolism in this species will enrich the theoretical basis for precise light environment regulation in medicinal plants. This study focuses on analyzing the mechanisms by which shading environments influence the growth and medicinal quality formation of *T. hemsleyanum*, aiming to provide scientific reference for its standardized cultivation, quality evaluation, and sustainable industrial development.

2 Ecological Habits and Shading Adaptation Basis of *Tetrastigma hemsleyanum*

2.1 Natural habitat and low-light adaptation characteristics of *Tetrastigma hemsleyanum*

Tetrastigma hemsleyanum is a perennial herbaceous climbing vine belonging to the genus *Tetrastigma* in the family Vitaceae, and it is one of the important medicinal plant resources in China. Its natural distribution is mainly concentrated in warm evergreen forests in subtropical and tropical regions of China. It is commonly found in understory habitats, forest edges, valleys, streamside areas, and hillside shrublands at altitudes of 300-1300 m, and may also extend to regions such as Hainan and Taiwan (Ren et al., 2025). From an ecological perspective, *T. hemsleyanum* prefers cool and humid environments, relatively high air humidity, loose soils rich in humus, and yellow or yellow-brown soils. Its natural habitats are generally characterized by sufficient scattered light, weak direct sunlight, and relatively stable hydrothermal conditions (Ji et al., 2020; Hu et al., 2021). This indicates that *T. hemsleyanum* is not a typical heliophilous plant, but is more suitable for growth in semi-shaded and humid environments with a certain degree of canopy cover. This also provides an ecological basis for its application in understory cultivation, trellis cultivation, and intercropping systems.

During long-term adaptation to understory or semi-shaded environments, *T. hemsleyanum* has developed a series of low-light adaptation characteristics. The plant mainly grows as a climbing vine and can extend outward by relying on surrounding vegetation or supports, thereby obtaining suitable scattered light resources. As the main photosynthetic organs, leaves are highly sensitive to changes in light conditions. Under low-light conditions, they can improve light interception capacity by regulating leaf area, chlorophyll content, and spatial leaf distribution. Ensemble habitat modeling further indicates that the current highly suitable habitats of *T. hemsleyanum* are mainly concentrated in subtropical regions jointly constrained by specific temperature and precipitation conditions, showing strong climatic adaptation specificity. Genomic and landscape genomic studies also show that *T. hemsleyanum* exhibits obvious local adaptation to heterogeneous climates. Winter precipitation and other climatic factors can explain a considerable proportion of its genomic variation, and many adaptive loci are associated with stress responses and environmental adaptation (Ren et al., 2025). Although direct measurements of the natural canopy light environment of *T. hemsleyanum* remain limited, its distribution in evergreen forests, preference for cool and humid environments, and the need to avoid strong light in bionic cultivation collectively support the view that it has a shade-adapted, low-light ecological strategy (Xu et al., 2018; Hu et al., 2021).

2.2 Fundamental role of light intensity in the growth and development of *Tetrastigma hemsleyanum*

Light is one of the most important ecological factors in the growth and development of *T. hemsleyanum*. It not only provides energy for photosynthesis, but also acts as an environmental signal involved in regulating plant morphogenesis, substance accumulation, and metabolic activities. Suitable light intensity is beneficial for improving leaf photosynthetic efficiency, promoting organic matter synthesis and transport, and providing a material basis for vine elongation, leaf expansion, and tuberous root enlargement. Shading experiments have shown that light intensity is a key factor regulating the growth, photosynthesis, and pigment accumulation of *T. hemsleyanum*. Under different shading levels, leaf size and net photosynthetic rate (Pn) reach relatively high levels under approximately 67%~70% shading, whereas stronger light and excessive shading both reduce plant growth and carbon assimilation capacity (Dai et al., 2009; Xu et al., 2018). This suggests that *T. hemsleyanum* has a clear suitable range of light intensity, and that either excessively strong or insufficient light is unfavorable for its sustained growth and formation of medicinal organs.

Both excessively high and excessively low light intensity may adversely affect *T. hemsleyanum*. Under strong light conditions, leaf transpiration increases and plant water consumption accelerates. When accompanied by high temperature and insufficient soil moisture, this may easily lead to leaf wilting, scorching, or photoinhibition, thereby reducing photosystem stability and photosynthetic efficiency. Studies have shown that under full sunlight and low-shading conditions, electron transport rate and photochemical quenching in *T. hemsleyanum* are inhibited, while non-photochemical quenching increases, indicating that excessively strong light can induce photoinhibition and reduce light-use efficiency. Conversely, under long-term excessive shading or insufficient low-light conditions, although *T. hemsleyanum* can enhance low-light capture capacity by increasing chlorophyll a, chlorophyll b, and total chlorophyll contents and reducing the chlorophyll a/b ratio, the accumulation of net photosynthetic products remains limited due to insufficient photosynthetically active radiation (Dai et al., 2009). Light response curve studies also indicate that the net photosynthetic rate of *T. hemsleyanum* increases rapidly under moderate photon flux density, then tends to become saturated, and decreases under high-light conditions, while the suitable light saturation point and maximum net photosynthetic rate mainly occur under moderate shading conditions (Xu et al., 2018). Therefore, the effect of light intensity on *T. hemsleyanum* is clearly dual in nature, and the key lies in maintaining a dynamic balance among light energy utilization, carbon assimilation, and stress alleviation.

2.3 Significance of shading environment for adaptation to artificial cultivation

The shading environment has important ecological regulatory significance in the artificial cultivation of *T. hemsleyanum*. Since the natural habitat of *T. hemsleyanum* is mostly semi-shaded and humid, exposing artificially cultivated plants completely to strong light may easily create a mismatch between their ecological niche requirements and the cultivation environment. In recent years, with the decline of wild *T. hemsleyanum* resources and increasing demand for medicinal materials, artificial cultivation has expanded rapidly, making light environment management a core issue for sustainable production. Based on the fact that *T. hemsleyanum* naturally grows in forest environments and is sensitive to strong light, bionic cultivation has been considered an important approach to meeting medicinal material demand and improving cultivation adaptability (Xu et al., 2018). By using shading nets, trellises, understory intercropping, or intercropping with tall-stemmed crops, direct light intensity can be reduced, the proportion of scattered light can be increased, and the field microclimate can be improved, thereby creating a growth environment close to its natural habitat. Relevant studies suggest that shading measures capable of achieving approximately 67%~70% shading may be adopted in cultivation, because higher light intensity inhibits its photosynthetic activity and growth, while excessive shading restricts carbon assimilation (Dai et al., 2009).

From the perspective of production practice, the value of shading treatment lies not only in promoting the growth of *T. hemsleyanum*, but also in regulating the relationship between yield and quality. Under suitable shading conditions, *T. hemsleyanum* can affect the formation and accumulation of active components such as flavonoids, polysaccharides, and phenolic compounds by improving leaf photosynthetic function, maintaining antioxidant system activity, regulating carbon-nitrogen metabolism, and promoting secondary metabolic processes. Studies on

other shade-tolerant medicinal plants also support this pattern, indicating that moderate shading can improve growth, photosynthetic performance, and secondary metabolite accumulation, whereas full sunlight and deep shading may both have adverse effects. For example, in medicinal plants such as *Eleutherococcus senticosus*, *Pinellia ternata*, *Paris polyphylla* var. *chinensis*, and *Polygala fallax*, moderate shading can increase chlorophyll content, optimize photosystem function, and increase biomass or certain active components, whereas excessively strong or weak light may lead to growth inhibition, light damage, or reduced accumulation of active components (Xu et al., 2020; Liang et al., 2022; Gao et al., 2025; Liu et al., 2026). Reviews on light-regulated secondary metabolism further indicate that precise control of light intensity and light quality is an important means of enriching valuable medicinal metabolites, which also highlights the importance of shading regimes in quality-oriented cultivation of *T. hemsleyanum* (Zhang et al., 2021).

Therefore, establishing a rational shading management model not only helps improve the adaptability of *T. hemsleyanum* to artificial cultivation environments, but also provides important technical support for high-quality, efficient, and standardized cultivation. Especially in medicinal plant cultivation, yield improvement does not necessarily equate to quality enhancement. How to achieve coordination among tuberous root yield, active component content, and commercial traits of medicinal materials is a core issue in optimizing *T. hemsleyanum* cultivation technology. In the future, shading cultivation of *T. hemsleyanum* should be based on a clear understanding of its ecological habits and photosynthetic adaptation mechanisms, and should further integrate shading intensity, light quality regulation, cultivation models, and quality evaluation indicators to establish a widely applicable standardized light environment management scheme, thereby supporting resource conservation, stable medicinal material quality, and industrial development of *T. hemsleyanum*.

3 Effects of Shading Environment on Morphogenesis and Biomass Accumulation of *Tetrastigma hemsleyanum*

3.1 Effects on plant height, vine growth, and branching

The shading environment first affects the aboveground morphogenesis of *Tetrastigma hemsleyanum*, especially in terms of plant height, vine elongation, and branch growth. As a perennial vine, *T. hemsleyanum* often grows in understory or semi-shaded and humid environments in the wild, where it receives scattered light filtered through the forest canopy. Therefore, its artificial cultivation also requires light regulation to approximate its natural ecological niche requirements as closely as possible (Ji et al., 2020). Vine growth capacity is directly related to spatial expansion, leaf distribution, and light resource acquisition in *T. hemsleyanum*. Under moderate shading conditions, plants can enhance their utilization of scattered light by increasing vine length, adjusting internode distance, and improving the spatial arrangement of leaves. This morphological change represents an adaptive response to low-light environments, which helps expand the photosynthetic area and improve the light interception efficiency of the plant canopy under limited light resources.

The effects of shading on vine growth in *T. hemsleyanum* vary significantly with shading intensity. Shading experiments have shown that under full sunlight or only 50% shading, photosynthetic electron transport and photochemical quenching in *T. hemsleyanum* are inhibited, while photosynthetic activity and plant growth decline, indicating that strong light conditions suppress overall plant growth. In contrast, when light is reduced to approximately 67%~70% shading, the net photosynthetic rate, light saturation point, and maximum photosynthetic rate are higher than those under weaker shading treatments, indicating that this shading level is more conducive to carbon assimilation and vegetative vine growth (Xu et al., 2018). However, if shading is excessive, such as reaching 75%-90% or higher, insufficient photosynthetically active radiation restricts carbon assimilation and slows growth (Dai et al., 2009). Therefore, plant height, vine elongation, and branch formation in *T. hemsleyanum* are jointly constrained by photoinhibition under strong light and insufficient carbon supply under deep shading, while moderate to relatively high shading is more favorable for vine extension and coordinated plant structure formation.

From the general perspective of plant responses to shading, reduced light or a decreased red/far-red ratio can induce shade-avoidance responses, manifested as stem and internode elongation, but often at the cost of reduced

branching and limited development of harvestable organs (Yang and Li, 2017). Similar phenomena have also been observed in leguminous plants, where shading can promote taller and thinner plants while reducing the number of lateral branches and aboveground biomass. For naturally shade-tolerant *T. hemsleyanum*, moderate shading is more likely to result in adaptive elongation and canopy optimization rather than simple etiolation. However, under excessive shading, excessive vine elongation, overly extended internodes, weak stems, and suppressed lateral branching may still occur, ultimately affecting leaf area formation and photosynthetic product accumulation. Therefore, in cultivation, “vine elongation” should not be simply equated with “good growth”; instead, branch number, leaf distribution, vine robustness, and underground tuberous root development should be comprehensively evaluated.

3.2 Effects on leaf morphology and leaf area expansion

Leaves are the main organs through which *T. hemsleyanum* performs photosynthesis and senses changes in the light environment. Therefore, changes in leaf morphology under shading conditions are important manifestations of its low-light adaptation. Leaf traits of *T. hemsleyanum* are sensitive to shading environments and reflect typical shade-leaf characteristics. As shading increases, chlorophyll a, chlorophyll b, and total chlorophyll contents increase, while the chlorophyll a/b ratio decreases, indicating adaptive adjustment of the light-harvesting antenna system to capture limited photons more efficiently under low-light conditions (Dai et al., 2009; Xu et al., 2018). In general, under moderately low-light environments, *T. hemsleyanum* can enhance light capture capacity by expanding individual leaf area, increasing total leaf area, and increasing photosynthetic pigment content. Studies have shown that leaves of *T. hemsleyanum* reach their largest size under approximately 67% shading, whereas full sunlight, 50% shading, and deep shading of approximately 90% all result in smaller leaves (Dai et al., 2009).

Leaf area expansion can increase the plant's absorption range of scattered light, helping compensate for the decrease in light intensity per unit leaf area. These changes are consistent with general patterns observed in shade-tolerant medicinal plants and forest plants, in which moderate shading, compared with no shading or excessive shading, is more conducive to increasing leaf area, improving seedling quality, and enhancing leaf function (Xue et al., 2023; Liu et al., 2026). In other shade-tolerant medicinal plants, low to moderate shading can thin the palisade tissue, increase total leaf thickness, and improve mesophyll structure, thereby promoting light absorption and carbon dioxide diffusion (Li et al., 2025). In *T. hemsleyanum*, increased chlorophyll content under approximately 67%~70% shading, together with improved net photosynthetic rate and other photosynthetic parameters, suggests that its leaf structure and function may also undergo optimization favorable for low-light utilization (Dai et al., 2009; Xu et al., 2018).

However, leaf area expansion does not necessarily indicate increased biomass accumulation. If shading intensity is too high, leaves may show certain shade-adaptive characteristics, such as deeper green color, increased chlorophyll content, or thinner leaf blades. Nevertheless, due to insufficient photosynthetically active radiation, net photosynthetic capacity per unit leaf area may decline, causing leaves to shift from highly efficient production organs to organs with relatively high maintenance costs. Long-term deep shading may also reduce leaf structural stability and stress resistance and increase the risk of disease occurrence. When shading is excessive, the decline in light saturation point and photosynthetic capacity in *T. hemsleyanum* limits carbon acquisition, thereby restricting leaf expansion and total leaf area formation (Xu et al., 2018). Therefore, the effects of shading on leaf morphology should be evaluated comprehensively from three aspects: leaf area expansion, leaf functional maintenance, and photosynthetic efficiency improvement. Among these, moderate shading regimes are more conducive to allowing *T. hemsleyanum* to fully express shade-leaf morphological potential, improve canopy light interception, and promote biomass accumulation.

3.3 Effects on tuberous root enlargement and biomass allocation

The main medicinal organs of *T. hemsleyanum* are enlarged spindle-shaped tuberous roots. These tuberous roots serve both as storage tissues and as pharmacologically active tissues, and their enlargement and dry matter accumulation are directly related to medicinal yield and commercial value (Figure 1) (Ji et al., 2020). The effect of shading environment on tuberous root formation essentially depends on the coordination between aboveground

photosynthetic product supply and underground assimilate allocation. At present, direct studies on the relationship between shading and tuberous root enlargement in *T. hemsleyanum* remain limited, and existing research has mainly focused on changes in aboveground photosynthesis under different light intensities (Hu et al., 2021). Current data indicate that unsuitable light environments, such as strong light, high temperature, and low humidity, reduce photosynthetic activity and slow plant growth in *T. hemsleyanum*, thereby indirectly limiting carbohydrate supply to the roots (Xu et al., 2018). In contrast, 67%~70% shading can maintain relatively high maximum net photosynthetic rate and quantum efficiency, creating favorable conditions for sustained assimilate production and its storage in tuberous roots (Dai et al., 2009).



Figure 1 The aerial part (A), root tuber (B) and raw herb (C) of *T. hemsleyanum*

Studies on other medicinal plants with storage organs help explain the possible biomass allocation patterns of *T. hemsleyanum* under different shading regimes. In shade-responsive species, moderate shading generally increases total biomass more effectively than full sunlight or deep shading, and may also increase root or storage organ biomass (Liang et al., 2022; Xue et al., 2023). Comprehensive studies of biomass allocation have shown that plants under environmental stress or resource limitation often allocate a relatively greater proportion of biomass to underground parts, thereby increasing the root-shoot ratio. However, this does not necessarily mean improved medicinal yield, because increased underground allocation may be accompanied by damage to aboveground photosynthetic structures and a decline in total biomass (Qi et al., 2019). In medicinal herbaceous plants, light shading may reduce the root-shoot ratio while increasing leaf biomass and total yield; in contrast, high light and dense planting may promote biomass allocation to roots, but at the expense of restricted aboveground growth (Ahmed et al., 2024). In storage tuber crops such as potato, simulated shading promotes stem elongation but reduces tuber yield, indicating that excessive shading may redirect assimilates from storage organs toward stem elongation (Gómez-Ocampo et al., 2023).

Therefore, from the perspective of medicinal material production, an ideal shading environment should not simply promote stem and leaf growth, nor should it simply increase the root-shoot ratio. Instead, it should maintain a dynamic balance between aboveground photosynthetic structure formation and underground medicinal organ accumulation. *T. hemsleyanum* likely has an optimal shading range close to the 67%~70% shading level identified in photosynthetic performance studies. Within this range, aboveground assimilation and underground storage processes can be well coordinated, thereby simultaneously supporting vigorous vine growth and effective tuberous root enlargement. In contrast, both strong light stress and excessively deep shading may lead to unfavorable biomass allocation and reduced medicinal yield. Therefore, in *T. hemsleyanum* cultivation, shading intensity should be reasonably controlled according to different growth stages so as to maintain good vegetative growth while ensuring tuberous root formation and medicinal yield improvement.

4 Effects of Shading Environment on Photosynthetic Physiology and Stress Resistance of *Tetrastigma hemsleyanum*

4.1 Effects on photosynthetic pigments and light-harvesting capacity

Photosynthetic pigments are an important material basis for light energy absorption, transfer, and conversion in *Tetrastigma hemsleyanum*, and changes in their content and composition directly reflect the plant's adaptability to shading environments. Shading treatment can significantly alter the pigment system of *T. hemsleyanum* and related shade-tolerant plants, thereby reshaping their light-harvesting capacity. Studies have shown that, in *T. hemsleyanum*, chlorophyll a, chlorophyll b, and total chlorophyll contents gradually increase with increasing shading intensity, while the chlorophyll a/b ratio decreases, indicating a typical shade-leaf adaptive expansion of the light-harvesting complex. This adjustment helps enhance the absorption of scattered and weak light under low-light conditions (Dai et al., 2009; Xu et al., 2018). In particular, the increase in chlorophyll b content is important for expanding the light-harvesting antenna system and improving light energy utilization efficiency under weak light. Similar phenomena have also been observed in other shade-managed crops, in which shading promotes the accumulation of chlorophyll and carotenoids and, compared with full sunlight, helps maintain greener leaves and a higher net photosynthetic rate (Elango et al., 2023).

In addition to chlorophylls, auxiliary pigments such as carotenoids also participate in light absorption, energy transfer, and photoprotection. Under suitable shading conditions, increases in chlorophyll and carotenoid contents can enhance light absorption capacity and improve the processing efficiency of absorbed light energy. At the same time, they reduce strong light-induced photooxidative pressure and decrease damage caused by excessive light energy accumulation in leaves. Shade-grown leaves usually show a reduced chlorophyll a/b ratio along with structural adjustments, which helps improve the utilization of transmitted light and avoid photoinhibition (Sagun et al., 2022). In overwintering tea plants and other shade-managed crops, shading can also upregulate genes related to chlorophyll and carotenoid metabolism as well as core photosystem protein genes, thereby enhancing light-harvesting capacity under low-light conditions (Simkin et al., 2022; Han et al., 2023). Therefore, moderate shading can enhance the adaptability of *T. hemsleyanum* to weak-light environments by optimizing pigment composition and the light-harvesting system. However, if shading is excessive, although leaves may exhibit apparent features such as deeper green color and increased chlorophyll content, insufficient incident light energy will still restrict light reactions and carbon assimilation, ultimately reducing photosynthetic production capacity.

4.2 Effects on photosynthetic efficiency and stomatal regulation

Photosynthetic efficiency is an important indicator for evaluating the growth potential of *T. hemsleyanum* under different light environments. Moderate shading can alleviate physiological stress caused by strong light, high temperature, and excessive water transpiration, helping maintain favorable stomatal opening and photosynthetic system activity. Across a series of shading treatments, the net photosynthetic rate, stomatal conductance, transpiration rate, light saturation point, and maximum net photosynthetic rate of *T. hemsleyanum* all increased with enhanced shading and reached relatively high levels under approximately 70% shading, before declining under deeper shading. Across different growth stages, the 70% shading treatment consistently showed superior photosynthetic characteristics (Xu et al., 2018). Another shading experiment also showed that under 67% shading, *T. hemsleyanum* had the largest leaves, the highest net photosynthetic rate, and a light saturation point of $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whereas stronger light and excessive shading both reduced carbon assimilation capacity and plant growth (Dai et al., 2009). These findings indicate that the photosynthesis of *T. hemsleyanum* exhibits an obvious "moderate optimum" response to shading intensity.

Shading also reshapes stomatal regulation and photochemical processes in *T. hemsleyanum*. Under suitable shading conditions, stomatal conductance and transpiration rate remain relatively coordinated, which helps ensure carbon dioxide entry into leaves for carbon assimilation. Meanwhile, the milder microclimate under shading can reduce leaf water deficit and decrease photosynthetic limitation caused by stomatal closure. Studies have shown that stomatal conductance and transpiration rate in *T. hemsleyanum* increase as shading rises to 70%, and then decline; unsuitable conditions such as strong light and high temperature reduce photosynthetic activity and slow plant growth (Xu et al., 2018). Under full sunlight and low-shading conditions, electron transport rate and

photochemical quenching in *T. hemsleyanum* decrease, while non-photochemical quenching increases, indicating the occurrence of photoinhibition. Moderate shading, by contrast, can maintain relatively high photochemical efficiency (Dai et al., 2009). Similar patterns have also been reported in other woody and medicinal plants: net photosynthetic rate is relatively high under moderate shading, the functions of photosystem II and photosystem I are more coordinated, whereas strong light or heavy shading may cause photosystem functional imbalance (Barazetti et al., 2021).

However, the effect of shading on photosynthetic efficiency is dual in nature. When shading is too strong, insufficient photosynthetically active radiation becomes the main factor limiting photosynthesis in *T. hemsleyanum*. Under such conditions, even if stomatal conductance does not decline significantly, insufficient light energy received by leaves will restrict both light and dark reactions, resulting in a decrease in net photosynthetic rate. Long-term heavy shading reduces organic matter synthesis, disrupts the balance between respiratory consumption and material accumulation, and further affects robust vine growth, leaf functional maintenance, and underground tuberous root enlargement. Therefore, the key to shading cultivation is not simply to reduce light exposure, but to regulate light intensity rationally so that *T. hemsleyanum* can avoid strong light stress while obtaining sufficient light energy to support biomass accumulation and medicinal quality formation.

4.3 Effects on the antioxidant system and cellular homeostasis

The shading environment also regulates stress resistance and cellular homeostasis in *T. hemsleyanum* by affecting the production and scavenging of reactive oxygen species. Regulation of the antioxidant system is an important mechanism by which plants cope with light stress. Although direct studies on antioxidant enzyme activities in *T. hemsleyanum* remain relatively limited, related studies in model shade-tolerant plants and medicinal plants are more abundant. Under strong light, high temperature, water stress, or other unfavorable light environments, the photosynthetic electron transport chain in leaves is prone to over-reduction, resulting in the accumulation of reactive oxygen species (ROS), which can further induce membrane lipid peroxidation, protein damage, and destruction of photosynthetic structures (García-Caparrós et al., 2020; Mishra et al., 2023). Therefore, plants must rely on enzymatic and non-enzymatic antioxidant systems to remove excess ROS, protect cellular structures, and maintain metabolic stability.

The main enzymatic antioxidant components include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR). These enzymes act cooperatively to convert reactive oxygen species such as superoxide anions and hydrogen peroxide into less harmful products. Meanwhile, non-enzymatic antioxidants such as ascorbic acid, glutathione, carotenoids, and flavonoids can also provide additional redox buffering capacity (García-Caparrós et al., 2020; Rajput et al., 2021). Moderate shading can reduce excessive light energy input, alleviate photooxidative pressure, and help maintain coordinated operation of antioxidant enzyme systems, thereby improving the plant's buffering capacity against environmental fluctuations.

Under different light conditions, many plants can maintain ROS homeostasis by upregulating antioxidant-related gene expression and increasing antioxidant enzyme activities. In the shade-tolerant plant *Solidago canadensis*, increased shading can induce high expression of SOD, POD, CAT, APX, and GPX genes, thereby enhancing ROS scavenging capacity under shading stress. In shade-tolerant *Panax notoginseng*, strong light stress increases SOD, POD, and CAT activities, while non-photochemical quenching dissipates excess energy to prevent photooxidative damage (Cun et al., 2023). Jasmine shows rapid changes in SOD, POD, APX, and CAT activities under different shading levels, and these changes are affected by both shading intensity and duration. Moderate shading generally supports more efficient antioxidant responses and reduces membrane lipid peroxidation compared with full sunlight or heavy shading (Deng et al., 2018). Based on these findings, it can be inferred that, in *T. hemsleyanum*, suitable shading environments may reduce excessive ROS production caused by strong light or extreme shading, support balanced antioxidant enzyme activity, and maintain membrane integrity, osmotic regulation capacity, and

redox homeostasis, thereby providing a physiological basis for enhanced stress resistance and stable medicinal quality formation.

5 Effects of Shading Environment on Substance Metabolism and Active Component Accumulation in *Tetragastria hemsleyanum*

5.1 Effects on carbohydrate accumulation and transport

Carbohydrates are an important material basis for the growth, development, and medicinal organ formation of *Tetragastria hemsleyanum*. Their accumulation level is directly affected by photosynthetic intensity, sugar metabolic activity, and the transport efficiency of assimilates. By altering the light intensity received by leaves, the shading environment further affects the synthesis, allocation, and transport of carbohydrates such as soluble sugars, sucrose, and starch. Studies on light-regulated sugar metabolism indicate that light intensity and light quality can influence starch granule formation, sucrose synthesis, and vascular transport, thereby determining the amount of carbon sources available for plant growth, storage organ development, and secondary metabolism. Under moderate shading conditions, leaves of *T. hemsleyanum* are expected to maintain relatively stable photosynthetic activity while reducing physiological consumption caused by strong light and high temperature, which is conducive to the continuous formation and effective transport of photosynthetic products. At this stage, leaves function as “source” organs and can provide sufficient carbon support for vine growth and underground tuberous root enlargement.

Carbohydrate accumulation is highly sensitive to shading intensity. If shading is excessive, insufficient photosynthetically active radiation restricts carbon assimilation, reducing the production of photosynthetic products in leaves and subsequently affecting sucrose transport to underground tuberous roots and the accumulation of storage substances. In medicinal underground bud plants such as *Bletilla striata* and *Bletilla ochracea*, moderate shading or medium light intensity can increase aboveground and tuber dry weight, net photosynthetic rate, and total polysaccharide content, whereas excessive shading leads to a significant decline in these indicators (Xu et al., 2024). Suitable light intensity can promote sucrose production in leaves and improve its transport efficiency to storage organs. In storage organs, sucrose is further converted into key intermediates such as sucrose-6-phosphate, fructose-6-phosphate, glucose-6-phosphate, GDP-mannose, and UDP-glucose, which subsequently participate in polysaccharide biosynthesis (Zhu et al., 2024; Zhu et al., 2025). Conversely, under low-light or long-term shading conditions, glycolysis, galactose metabolism, the pentose phosphate pathway, and the tricarboxylic acid cycle are inhibited in many plants, resulting in reduced sugar reserves and causing plants to shift toward consuming stored carbohydrates (Liu et al., 2020; Shao et al., 2022). Therefore, the regulation of carbon metabolism by shading in *T. hemsleyanum* is not simply promotive or inhibitory; rather, it depends on whether shading intensity can maintain a balance among photosynthesis, respiratory consumption, and assimilate transport.

5.2 Effects on nitrogen metabolism and carbon-nitrogen balance

Nitrogen metabolism is an important basis for vegetative growth and physiological function maintenance in *T. hemsleyanum*, and it is closely associated with chlorophyll synthesis, protein formation, enzyme activity regulation, and secondary metabolism. Under shading conditions, *T. hemsleyanum* often needs to adjust leaf structure and photosynthetic pigment composition to adapt to weak-light environments, while chlorophyll, Rubisco, and other photosynthesis-related proteins all require nitrogen for their formation. General studies on carbon-nitrogen regulation have shown that increasing nitrogen allocation in leaves can enhance chlorophyll and Rubisco contents, photosynthetic nitrogen-use efficiency, and carbon assimilation capacity, and can help improve plant growth and carbon storage even under nitrogen-limited conditions (Perchlik and Tegeder, 2018). Therefore, under moderate shading conditions, *T. hemsleyanum* may enhance light absorption and utilization under weak light by optimizing nitrogen allocation for leaf functional maintenance and photosynthetic system construction, thereby providing a metabolic basis for subsequent substance synthesis and medicinal component accumulation.

Shading can also reshape nitrogen metabolism and carbon-nitrogen (C-N) balance, both of which jointly determine photosynthetic capacity, vegetative growth, and secondary metabolite biosynthesis in plants. In tea

plants, short-term shading can promote leaf nitrogen metabolism and increase amino acid accumulation, whereas long-term or high-intensity shading inhibits sugar metabolism and alters flavonoid metabolic pathways (Li et al., 2020). Under shading conditions, protein hydrolysis and nitrogen redistribution can increase free amino acid content, while catechin and other phenolic compound levels decline, reflecting a shift in carbon-nitrogen allocation direction (Shao et al., 2022). Shading experiments in forest plants have shown that, as shading increases, the leaf C:N ratio decreases while the N:P ratio increases, and non-structural carbohydrates are closely associated with C: N: P stoichiometric characteristics, suggesting a dynamic trade-off between carbon storage and nutrient utilization under low-light environments (Liu et al., 2020). For *T. hemsleyanum*, if shading is appropriate, carbon supply and nitrogen utilization can remain relatively coordinated, which is beneficial for protein synthesis, enzymatic reactions, and normal operation of metabolic pathways. If shading is excessive, however, carbon assimilation becomes restricted while nitrogen metabolic demand remains, potentially causing C-N imbalance and affecting plant dry matter accumulation and substrate supply for secondary metabolism.

5.3 Effects on the accumulation of flavonoids, polysaccharides, and phenolic compounds

Flavonoids, polysaccharides, and phenolic acids are important active components for evaluating the medicinal quality of *T. hemsleyanum*, and they are also representative pharmacologically active substances in its tuberous roots and leaves (Hu et al., 2021). The accumulation of these components is jointly regulated by multiple factors, including light intensity, spectral composition, temperature, water availability, nutrient status, and growth stage. The shading environment can influence the operation of secondary metabolic pathways in *T. hemsleyanum* by altering light signal input, photosynthetic product supply, and cellular redox status. Moderate shading can alleviate strong light stress and maintain a relatively stable physiological metabolic state, thereby providing a favorable cellular environment for active component synthesis. Meanwhile, suitable weak light or specific light quality stimulation may also induce plants to adjust phenylpropanoid metabolism and flavonoid biosynthesis, thereby affecting the accumulation of flavonoids and phenolic compounds.

Different active components do not respond uniformly to shading environments. In *T. hemsleyanum* cultivated under different colored films, blue film promoted vegetative growth and soluble amino acid accumulation, whereas red film significantly increased flavonoid content and the activity of key enzymes such as phenylalanine ammonia-lyase (PAL). This indicates that changes in light quality under shading environments may lead plants to exhibit different metabolic orientations between yield and quality (Bai et al., 2021). Long-term low-intensity blue light treatment of *T. hemsleyanum* tuberous roots can simultaneously increase tuberous root yield and total flavonoid content, enhance antioxidant activity, and upregulate genes related to flavanol biosynthesis (Zhao et al., 2024). In addition, seasonal analysis has shown that flavonoid content, as well as the antioxidant activities of major phenolic compounds and polysaccharides in *T. hemsleyanum*, fluctuate with changes in sunshine duration, temperature, and humidity, while suitable shading conditions help enhance the accumulation of medicinally relevant phenolic compounds and polysaccharides (Figure 2) (Shi et al., 2022). These results indicate that the accumulation of active components in *T. hemsleyanum* is sensitive to both light intensity and spectral composition, and shading management affects not only yield but also the intrinsic quality of medicinal materials.

Polysaccharide accumulation is usually closely related to carbohydrate metabolism, tuberous root development, and storage substance formation. Therefore, when excessive shading causes carbon source insufficiency, polysaccharide accumulation may be inhibited. Shading studies on *Bletilla* species have shown that moderate shading or medium light intensity can result in relatively high polysaccharide content, accompanied by increases in precursor substances such as sucrose-6-phosphate and glucose-6-phosphate. In contrast, excessive shading or strong light reduces polysaccharide levels and disrupts carbon metabolism (Xu et al., 2024; Zhu et al., 2024). Flavonoids and phenolic compounds are more strongly affected by light signals, oxidative stress, and secondary metabolic enzyme activities. Studies on tea plants have shown that strong shading generally inhibits flavonoid and catechin biosynthesis while increasing free amino acid content, indicating that reduced light can alter the balance between nitrogen-rich and carbon-rich metabolites (Li et al., 2020; Shao et al., 2022). Therefore, the effects of shading environments on the medicinal quality of *T. hemsleyanum* are component-specific, light quality-sensitive, and intensity-dependent. An ideal shading regime should promote the coordinated accumulation of major active

components such as flavonoids, polysaccharides, and phenolic compounds while ensuring tuberous root yield, thereby achieving simultaneous improvement in medicinal yield and intrinsic quality.

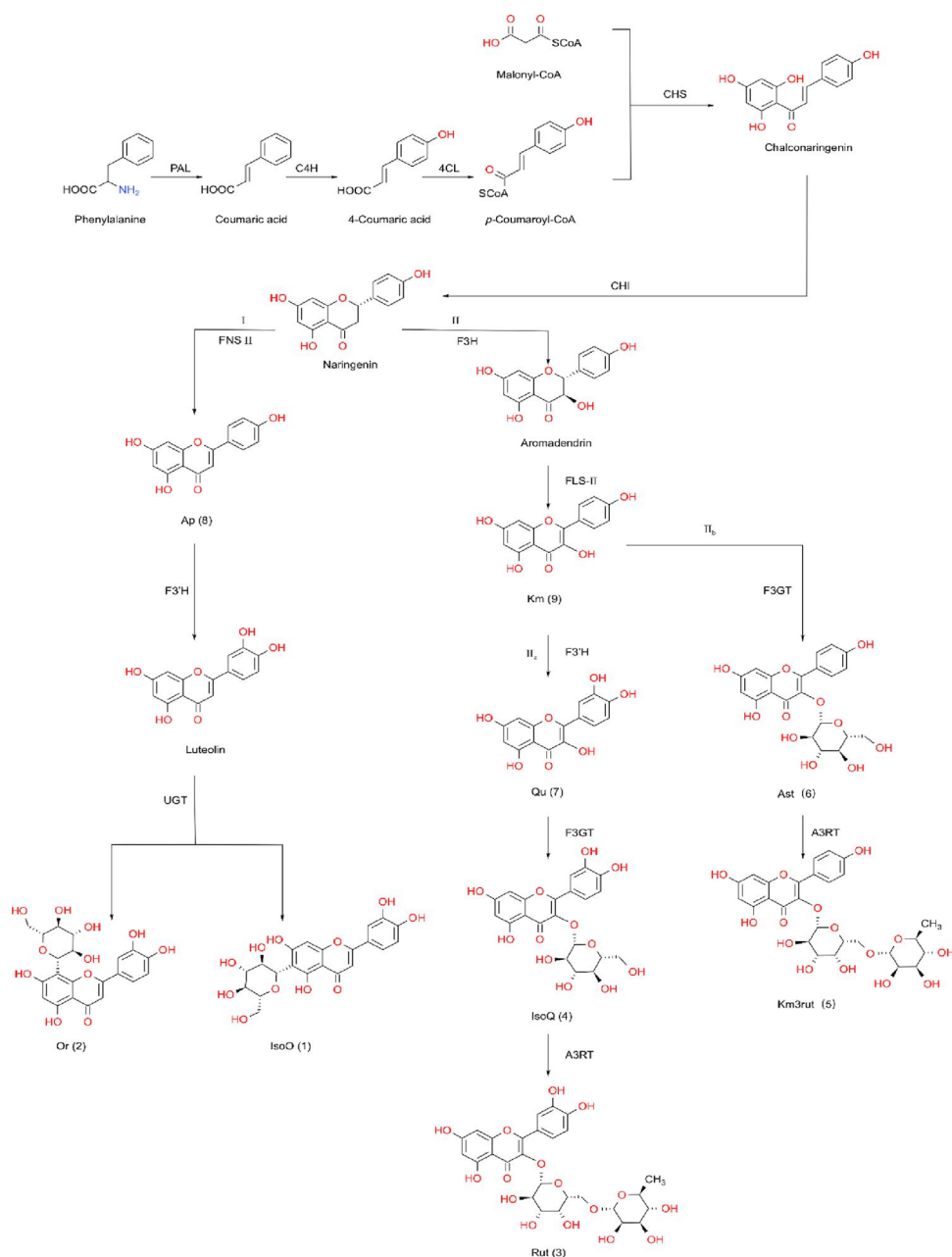


Figure 2 A schematic outline of flavonoid pathway proposed for *T. hemsleyanum* (Adopted from Shi et al., 2022)

Image caption: PAL, phenylalanine ammonia-lyase; C4H, trans-cinnamate 4-monooxygenase; 4CL, 4-coumarate-CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; FNS II, flavone synthase II; F3'H, flavonoid 3'-hydroxylase; F3H, flavonone 3-hydroxylase; FLS-II, flavone synthase II; UGT, UDP-glucosyltransferase; F3GT, flavonol-3-O-glucosyltransferase; A3RT, anthocyanidin-3-O-glucoside-6-O-rhamnosyltransferase (Adopted from Shi et al., 2022)

6 Mechanisms by Which Shading Regulates Medicinal Quality Formation in *Tetrastigma hemsleyanum*

6.1 Light signal perception and transduction mechanisms

Shading simultaneously alters light intensity and spectral composition, such as reducing the proportions of UV-B, blue light, and red light while enriching far-red light. Therefore, *Tetrastigma hemsleyanum* is expected to perceive shading environments through a series of photoreceptors similar to those identified in other plant species. Under canopy or shaded conditions, plants use phytochromes to perceive red and far-red light, cryptochromes and phototropins to perceive blue light/UV-A, and UVR8 to perceive UV-B, thereby recognizing changes in light

quality and quantity and correspondingly regulating morphology and metabolism (Liu et al., 2018). These photoreceptors converge on core regulatory hubs such as the COP1-SPA E3 ubiquitin ligase complex and the bZIP transcription factor HY5, integrating multi-wavelength signals and reprogramming gene expression (Tissot and Ulm, 2020).

In shaded tea plants, UVR8-, HY5-, and *COP1-related* genes show co-expression relationships with flavonoid biosynthesis genes, and their expression levels decrease synchronously with catechin and flavonol contents, indicating that weakening of UV-B and its related signaling directly affects secondary metabolism (Liu et al., 2018). More broadly, different wavelengths of light can activate HY5 and related factors through UVR8, cryptochromes, and phytochromes, thereby inducing the synthesis of phenylpropanoid “sunscreen” metabolites and other protective metabolites (Leonardelli et al., 2024). Therefore, in *T. hemsleyanum*, shading-induced changes in the UVR8-HY5-COP1 signaling pathway are likely to be key upstream mechanisms regulating the accumulation of medicinal phenylpropanoid and flavonoid compounds.

6.2 Regulatory mechanisms of secondary metabolic pathways

Light regulates the biosynthesis of phenolic compounds, terpenoids, alkaloids, and other substances through conserved transcriptional regulatory networks. Specific photoreceptors can activate signaling cascades and regulate the expression of genes involved in phenylpropanoid and flavonoid metabolic pathways, such as PAL, C4H, 4CL, CHS, CHI, F3H, F3'H, FLS, DFR, ANS, ANR, and LAR, thereby leading to tissue- and condition-specific accumulation of catechins, flavonols, anthocyanins, and other metabolites (Liu et al., 2023). In tea plants, shading treatment, namely 20%-25% light transmittance, reduces catechin and flavonol contents by more than 40%-50%, accompanied by coordinated downregulation of multiple flavonoid structural genes and UVR8 pathway components, directly demonstrating a link between shading and reduced phenylpropanoid metabolic flux (Liu et al., 2018).

Comprehensive reviews indicate that the effect of shading on medicinal quality depends on species shade tolerance and light level: moderate shading can increase total phenols, flavonoids, and specific compounds, whereas deep shading usually downregulates genes related to phenylpropanoid and terpenoid biosynthesis and reduces metabolite accumulation. Reviews of phenylpropanoid metabolism emphasize that this pathway generates thousands of metabolites, including flavonoids and phenolic acids with strong antioxidant and defensive functions, and that it is strictly regulated at both transcriptional and post-transcriptional levels to adapt to changing environmental conditions (Deng and Lu, 2017; Ninkuu et al., 2025). For *T. hemsleyanum*, its key medicinal components mainly include flavonoids and related compounds derived from the phenylpropanoid pathway. Therefore, shading is expected to reshape metabolic pathway flux by altering photoreceptor signaling and the expression of core biosynthetic genes.

6.3 Regulation of key enzyme genes and transcription factors

Light signals regulate medicinal quality not only through structural genes, but also through complex transcription factor networks. Core light-responsive regulators such as HY5, PIFs, BBX proteins, and COP1 can integrate photoreceptor input signals and interact with transcription factors such as MYB, bHLH, WRKY, bZIP, and NAC, thereby directing the expression of genes related to secondary metabolism (Liu et al., 2023; Chen et al., 2025). In shaded tea plants, photoreceptor genes and UVR8-HY5 pathway genes show co-expression relationships with MYB12, MYB4, and MYB111, supporting a model in which light-regulated MYB transcription factors control catechin and flavonol biosynthesis (Liu et al., 2018). In medicinal and horticultural plants, MYB, bHLH, WRKY, and related transcription factors serve as “master switches” of phenylpropanoid and flavonoid metabolic pathways and respond strongly to changes in the light environment. In yam, light exposure can increase anthocyanin and flavonoid contents, and light-induced MYB and WRKY transcription factors are highly correlated with anthocyanin metabolites and structural gene expression.

In pear, the light-responsive WRKY transcription factor PpWRKY44 is activated by BBX18 and can directly bind to the PpMYB10 promoter, thereby driving anthocyanin accumulation (Alabd et al., 2022). Multi-omics studies further show that blue light can upregulate MYB transcription factors as well as key structural genes such as PAL,

CHS, CHI, F3H, and FLS, thereby enhancing flavonoid accumulation. Reviews on medicinal plants also point out that the MYB, WRKY, bHLH, AP2/ERF, and NAC families are core regulatory factors mediating stress- and light-induced secondary metabolite biosynthesis (Tong et al., 2024; Rabeh et al., 2025). Based on these conserved mechanisms, it can be inferred that shading effects in *T. hemsleyanum* are likely mediated by regulating light-signaling hubs such as UVR8-COP1-HY5, which further control MYB/WRKY/bHLH regulatory networks and the expression of key enzyme genes in phenylpropanoid and flavonoid metabolic pathways, ultimately influencing medicinal quality formation.

7 Optimization Strategies for Shading Cultivation of *Tetrastigma hemsleyanum*

7.1 Reasonable determination of shading intensity

Physiological studies on *Tetrastigma hemsleyanum* provide clear evidence for determining an appropriate shading level. Under five light intensity treatments ranging from full sunlight to 90% shading, photosynthetic pigments and most photosynthetic indicators increased with increasing shading intensity and reached their peak at approximately 70% shading before declining. This indicates that 70% shading can maintain the highest net photosynthetic rate (Pn), maximum photosynthetic rate (Pmax), light saturation point, and stomatal conductance during both the rapid and slow growth stages (Xu et al., 2018). Another experiment conducted under full sunlight, 50% shading, 67% shading, and 90% shading showed that 67% shading produced the largest leaves and the highest photosynthetic rate. Light stronger than that under 50% shading inhibited photosynthesis and growth, whereas irradiance below that under 75% shading restricted carbon assimilation (Dai et al., 2009). Together, these findings suggest that approximately 65%-70% shading can serve as a reasonable baseline for field and greenhouse cultivation.

Experience from other medicinal plants also supports this principle, namely that moderate shading or medium light intensity can usually optimize both biomass and active component accumulation. In the shade-loving medicinal plant *Panax notoginseng*, the combination of three-layer shading, an appropriate red-blue light ratio, and moderate soil moisture produced the highest biomass and saponin content; in contrast, light under single-layer shading was still “excessive” relative to its physiological requirements and could damage photosynthesis and secondary metabolism. In *Pinellia ternata*, 55% full sunlight treatment increased chlorophyll content, photosynthetic rate, and succinic acid content compared with full sunlight, with succinic acid increasing by 27%, indicating that moderate shading can improve medicinal quality (Gao et al., 2025). Meanwhile, excessive shading, such as 72%-90% shading, may significantly reduce biomass and active component content in certain species (Deng et al., 2024). Therefore, for *T. hemsleyanum*, using 65%-70% shading as the core target and making slight adjustments within this range according to local light climate and quality objectives is a relatively reasonable cultivation strategy.

7.2 Stage-specific light environment regulation

The light requirements of *T. hemsleyanum* differ among developmental stages, and therefore a dynamic shading strategy is needed. The photosynthetic indicators of this species during the rapid growth stage are 41%-67% higher than those during the slow growth stage, yet 70% shading remains suitable in both stages. This suggests that, while maintaining the same basic shading intensity, other factors such as temperature and humidity can be further adjusted (Xu et al., 2018). However, studies on other herbaceous medicinal plants indicate that using full sunlight or relatively high light intensity at the early growth stage, followed by increased shading or spectral adjustment at later stages, may better coordinate biomass accumulation and quality formation. In *Origanum majorana*, 100% light produced the highest dry weight, whereas essential oil content peaked under 70% light; therefore, researchers suggested using high light during the early growth stage and 70% light transmittance shading treatment during the later growth cycle (Hashemifar et al., 2024).

Studies on understory medicinal plants and shade-tolerant plants further emphasize the importance of stage-specific light quality regulation. In *Scutellaria baicalensis*, compared with UV-A, green light, or mixed blue-red light treatments, monochromatic blue light (R0B4) better promoted growth and flavonoid accumulation, indicating that enhancing the blue-light component during stages targeting flavonoid synthesis may improve

quality. For *T. hemsleyanum*, when cultivated under different colored films at 70% total light transmittance, blue film (BF) was most favorable for promoting growth and yield formation, whereas red film (RF) was most effective in increasing PAL activity and flavonoid content (Bai et al., 2021). These results suggest that, on the basis of relatively stable 65%~70% shading, blue-enriched light spectra may be used during the vegetative expansion stage, while red-enriched spectra or films may be applied during the later medicinal quality formation stage to achieve stage-specific coordination between growth promotion and quality enhancement.

7.3 Optimization of cultivation models and quality evaluation systems

In addition to adjusting shading nets and spectral composition, optimizing cultivation models is also important for stabilizing the yield and quality of *T. hemsleyanum*. Comparative studies of different planting bases and cultivation modes have shown that greenhouse cultivation produces the thickest stems, longest leaves, and greatest aboveground and underground biomass, and that the total flavonoid content in tuberous roots under greenhouse cultivation is higher than that under understory cultivation. Under greenhouse conditions, the stereoscopic cultivation mode further increases the contents of indicator compounds, including polydatin, resveratrol glycoside, resveratrol, and kaempferol, compared with creeping cultivation (Hu et al., 2023). These findings suggest that “greenhouse + stereoscopic structure + optimized shading” may be a comprehensive cultivation model with strong potential for industrial production of *T. hemsleyanum*.

To support the above cultivation models, a systematic quality evaluation system and light regulation decision-making tools are also needed. A review on *T. hemsleyanum* pointed out that a scientific, universal, and measurable quality control system is still lacking, and called for the establishment of quality markers related to antitumor and anti-inflammatory activities (Hu et al., 2021). In other medicinal plants, comprehensive evaluation methods such as entropy-weighted TOPSIS have been used to screen combinations of red-blue light ratios, field water-holding capacity, and shading treatments, and to identify optimal cultivation conditions by comprehensively scoring growth, photosynthesis, and saponin content. Similar multi-index evaluation models can also be applied to *T. hemsleyanum*, using biomass and key components, such as flavonoids, resveratrol-like compounds, and polysaccharides, as core indicators. Combined with controlled shading regimes, such models can help establish standardized cultivation protocols, guide the construction of regional planting models, and support traceable quality evaluation from field production to market circulation.

8 Conclusion and Prospects

Existing studies clearly indicate that the light environment is a core ecological factor determining the growth performance and medicinal quality of *Tetrastigma hemsleyanum*. As an understory vine adapted to shaded environments, *T. hemsleyanum* exhibits systematic changes in photosynthetic pigments, gas exchange, and light-response parameters along shading gradients; unsuitable conditions such as strong light, high temperature, and low humidity can reduce photosynthetic activity and slow plant growth. Light quality is equally important. Under different colored film treatments, blue film can promote vegetative growth and yield formation, whereas red film enhances PAL activity and flavonoid accumulation, directly indicating that spectral composition is closely associated with biomass formation and active component accumulation. At the cultivation system level, compared with understory cultivation, greenhouse cultivation with a stereoscopic structure can significantly increase aboveground and underground biomass and improve total flavonoid content in tuberous roots, further confirming that integrated light environment regulation is a key approach for simultaneously improving yield and medicinal quality.

Multiple experiments have shown that moderate shading is the most effective strategy for coordinating vegetative growth, photosynthetic performance, and active component accumulation in *T. hemsleyanum* and similar shade-tolerant medicinal plants. In *T. hemsleyanum*, photosynthetic pigments and most photosynthetic indicators increase with enhanced shading and reach maximum values under approximately 70% shading during both rapid and slow growth stages; thereafter, excessive shading reduces photosynthetic capacity. Therefore, 70% shading has been considered suitable for different growth stages. Similarly, 67% shading results in the highest leaf area and net photosynthetic rate, whereas light stronger than that under 50% shading inhibits photosynthesis, and

irradiance below that under 75% shading restricts carbon assimilation and plant growth. This suggests that an appropriate window may exist near approximately two-thirds shading. From broader research on medicinal plants, moderate reduction of light intensity or optimization of scattered light proportion generally improves biomass and key metabolite contents, whereas excessively strong or weak light impairs survival, photosynthesis, and secondary metabolism. This further indicates that precise calibration of shading conditions is necessary to achieve the dual goals of high yield and stable medicinal quality.

Although existing studies have clarified the suitable shading range for *T. hemsleyanum* and demonstrated that light quality and cultivation mode strongly affect its growth and flavonoid accumulation, research on its intrinsic mechanisms and standardized application remains limited. Recent reviews have pointed out that current physiological studies of *T. hemsleyanum* mainly focus on light and fertilizer effects, with relatively few investigations into deeper regulatory processes or systematic quality control. They also emphasize that this species still lacks a scientific, universal, and measurable quality evaluation system. In contrast, integrated phenotypic, physiological, and transcriptomic analyses of other medicinal plants under light gradients have revealed how moderate light reallocates carbon sources, reshapes membrane structures, and directs metabolic flux toward photoprotection and bioactive compound production, providing a useful theoretical framework for mechanistic studies of *T. hemsleyanum*. Therefore, future research should integrate omics-based analyses of light signaling, carbon-nitrogen metabolism, and secondary metabolic networks with multifactorial cultivation experiments involving shading intensity, spectrum, cultivation mode, nutrient management, and water management. Multi-index evaluation or decision-making models should also be applied to construct standardized and scalable shading cultivation systems and quality evaluation systems suitable for resource conservation and industrial development of *T. hemsleyanum*.

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Research Insight

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Technical Pathways for Tissue Culture Rapid Propagation and Improvement of Transplant Survival Rate in *Dendrobium officinale*

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Abstract *Dendrobium officinale* is an important medicinal orchid plant in China with both medicinal and food uses. It has high medicinal value, health-care value, and industrial development potential. However, its wild resources are limited, and the efficiency of conventional propagation is relatively low, making it difficult to meet the demand for large-scale and standardized seedling production. Tissue culture rapid propagation provides an important technical pathway for the rapid multiplication of high-quality *D. officinale* seedlings and the conservation of germplasm resources. This study explored technologies for tissue culture rapid propagation and improvement of transplant survival rate in *D. officinale*, with emphasis on key links including explant selection, establishment of an aseptic culture system, optimization of culture media and nutritional conditions, protocorm induction and subculture proliferation, multiple shoot differentiation, rooting and strong seedling cultivation, seedling grading before bottle removal, hardening-off acclimatization, and transplant management. The results indicate that the rapid propagation efficiency of *D. officinale* is jointly affected by explant type, basal medium, plant growth regulators, organic additives, activated carbon, and subculture cycle. Meanwhile, transplant survival rate is closely related to root quality, seedling vigor, acclimatization intensity, substrate formulation, temperature and humidity management, and beneficial microbial interactions. By constructing a continuous technical chain of “induction-proliferation-rooting-strong seedling cultivation-hardening-off-transplanting,” seedling uniformity, transplant adaptability, and production stability can be effectively improved. In the future, further efforts should be made to strengthen research on the standardization of *D. officinale* tissue culture systems, seedling quality evaluation, intelligent management, and microbial synergistic regulation, thereby providing technical support for the industrialized, standardized, and sustainable development of *D. officinale*.

Keywords *Dendrobium officinale*; Tissue culture rapid propagation; Protocorm; Strong seedling cultivation; Transplant survival rate

1 Introduction

Dendrobium officinale Kimura et Migo, also known as *Dendrobium catenatum*, is a precious medicinal plant of Orchidaceae that occupies an important position in traditional Chinese medicine, functional foods, and the modern health industry (Li et al., 2024; Wei et al., 2024). It has been used in China for thousands of years, and its dried stems are officially recorded in the Chinese Pharmacopoeia and included in the catalogue of substances with both medicinal and food uses, reflecting both long-standing traditional application and broad consumption potential (Zhang et al., 2023; Wei et al., 2024). Traditional records describe its functions in nourishing yin, benefiting the stomach, promoting fluid production, and supporting general health, while modern studies further show that *D. officinale* contains diverse bioactive constituents including polysaccharides, bibenzyls, flavonoids, phenanthrenes, alkaloids, and other phenolics (Duan et al., 2022; Zhang et al., 2023). Reviews have reported that these constituents are associated with antioxidant, anti-inflammatory, immunomodulatory, gastrointestinal protective, hepatoprotective, hypoglycemic, neuroprotective, and antitumor activities, which explains why *D. officinale* has become one of the most intensively studied medicinal species in the genus (Xu et al., 2022). At the broader genus level, China has abundant *Dendrobium* resources, with dozens of medicinally valuable taxa and at least 78 species reported nationally, but *D. officinale* remains one of the most prominent research and development targets because of its recognized medicinal value, edible potential, and market appeal. In addition, the plant has expanding value beyond stems alone: recent work indicates that leaves and flowers also contain numerous active compounds and

can be developed for food, health-care, and nutraceutical uses, suggesting that the industrial chain of *D. officinale* is still widening.

The rising medicinal, nutritional, and commercial demand for *D. officinale* has sharply increased pressure on germplasm resources and seedling supply. Because wild populations were overexploited, the species has been listed as a secondary endangered plant in China, and rational protection together with artificial propagation has become a central issue in its utilization. Although artificial cultivation technology has made progress, current cultivation resources are still mixed, product quality is uneven, and the evaluation system remains imperfect, all of which constrain standardized industrial development. Conventional propagation routes are not sufficient to meet large-scale market demand because they depend on limited source materials and generally cannot provide rapid, uniform, and continuous production of elite seedlings (Xu et al., 2022). This limitation is reflected indirectly by later rapid-propagation studies, which were specifically designed to solve restricted explant availability, low propagation efficiency, and the need for large-batch production. Orchid micropropagation research more broadly identifies low shoot multiplication, clonal instability, poor rooting frequency, and high production cost as major obstacles in medicinal orchid propagation, indicating that propagation efficiency and seedling quality are persistent technical barriers rather than trivial operational details. For *D. officinale* specifically, the transition from in vitro culture to greenhouse or field conditions is another key bottleneck, because tissue-cultured seedlings often show low survival and weak growth after transplanting. This means that even when aseptic seedlings can be produced in vitro, industrial propagation still fails if rooting, hardening-off, substrate selection, microbial symbiosis, and transplant management are not optimized into an integrated technical chain (Wang, 2021; Li et al., 2024).

For that reason, recent studies on *D. officinale* tissue culture have shifted from simple seedling induction toward the construction of complete rapid-propagation systems covering explant disinfection, adventitious bud or protocorm induction, proliferation, differentiation, strong-seedling culture, rooting, hardening, and substrate transplanting. Several reports show that appropriate combinations of basal medium, plant growth regulators, activated carbon, and organic additives such as banana juice, potato extract, or coconut juice can markedly improve shoot multiplication, rooting, and seedling vigor. Likewise, transplant survival is highly sensitive to the hardening process and to the choice of substrate (Li et al., 2023). Reported survival outcomes vary by protocol, from 80% in peat-pine bark-macadam mixtures, to 88% in fine wood substrate, to 95% after sphagnum hardening-off and 97% after matrix transplantation, while some planting methods under optimized cultivation management report survival above 98%. Other work suggests that biological assistance can further improve acclimatization, since *Mycena dendrobii* enhanced seedling survival and growth by promoting stress tolerance and new root formation (Wei et al., 2024). Meanwhile, highly efficient protocorm-like body systems have reported 99% induction, a differentiation coefficient of 50 seedlings per 0.1 g protocorm-like bodies, 100% rooting, and transplant survival above 95%, highlighting the feasibility of industrialized seedling production once the full pathway is optimized.

This study will explore the technical pathways for tissue-culture rapid propagation and improvement of transplant survival rate in *Dendrobium officinale*, and systematically sort out the key links affecting propagation efficiency and post-bottle establishment, including explant selection, medium screening, hormone regulation, rooting enhancement, hardening-off, substrate configuration, and transplant management. The significance of this study lies not only in increasing the multiplication coefficient and shortening the seedling production cycle, but also in stabilizing seedling quality, improving the survival rate of tissue-cultured plantlets, and providing a technical foundation for standardized and large-scale cultivation. From a broader perspective, efficient rapid propagation technology can reduce dependence on wild resources, support the conservation and sustainable utilization of this endangered medicinal orchid, and better meet the growing demand from the pharmaceutical, functional food, and health-product industries. Since pharmacological development, product diversification, and quality control all depend on a stable and sufficient supply of raw materials, establishing reliable rapid-propagation technology and

high-survival transplant technology is a necessary prerequisite for the modern industrialization and high-value utilization of *Dendrobium officinale*.

2 Technical Basis of Tissue Culture Rapid Propagation of *Dendrobium officinale*

2.1 Selection of explants of *Dendrobium officinale*

The choice of explant is the first determinant of rapid propagation efficiency in *Dendrobium officinale*, because explant type affects contamination risk, induction rate, regeneration pathway, and the uniformity of resulting seedlings. Current *Dendrobium* aseptic culture protocols are strongly tissue- and genotype-dependent, so explant selection cannot be separated from the later sterilization and culture scheme (Sukmadjaja and Widhiastuti, 2019). In *D. officinale*, reported explants include seeds, stem segments with nodes, budding stems, stem tips, leaves, and protocorm-like bodies, indicating that the species has multiple available regeneration routes (Nguyen et al., 2022). For large-scale initiation, seeds are especially important because they are abundant and can directly enter asymbiotic germination and protocorm induction systems (Mamun et al., 2018).

Comparative evidence indicates that seeds are often the most suitable explant for protocorm and PLB induction in *D. officinale*. One study comparing five explant types reported a 100% induction rate and the best growth status from seeds, while leaves, stem fragments, stem tips, and stems with nodes showed lower induction rates of 43%, 25%, 52%, and 31%, respectively. However, for clonal multiplication of selected germplasm, nodal and stem-derived explants have clearer advantages because they preserve elite genotypes and support uniform vegetative propagation (Nguyen et al., 2022). Stem segments with nodes were reported to achieve 93.3% bud induction on 1/2 MS supplemented with 1.5 mg/L 6-BA and 0.5 mg/L NAA, while budding stems have also been used to establish complete rapid-propagation systems (Silva et al., 2017). Therefore, seed explants are more suitable for high-frequency initiation and PLB establishment, whereas nodal or budding-stem explants are more suitable for stable clonal propagation of target lines (Mamun et al., 2018).

2.2 Establishment of an aseptic culture system for *Dendrobium officinale*

The establishment of an aseptic culture system is the technical prerequisite for all subsequent stages of *D. officinale* micropropagation, because contamination at culture initiation directly reduces explant survival and later regeneration success. In *Dendrobium*, aseptic culture success depends on the source, size, age, and physiological state of explants, as well as the preparation and disinfection procedure used before inoculation. Most donor materials are obtained from greenhouse or similar non-sterile environments, so surface sterilization is indispensable before explants can be transferred into in vitro culture (Sukmadjaja and Widhiastuti, 2019). Because different tissues respond differently to disinfectants, no single sterilization procedure is universally optimal for all *Dendrobium* explants or genotypes.

For *D. officinale*, ethanol combined with mercuric chloride remains one of the most commonly reported effective sterilization schemes for stem-derived explants. A systematic rapid-propagation study found that 75% ethanol for 30 s followed by 0.1% HgCl₂ for 10 min was the best disinfection method for budding stems (Silva et al., 2017). Supporting evidence from another *Dendrobium* nodal-segment study showed that 0.1% HgCl₂ for 10 min produced the lowest contamination rate and the highest healthy culture establishment, while stronger sodium hypochlorite treatment increased explant mortality. In seed culture, aseptic initiation is often simplified by collecting capsules near maturity and releasing seeds directly under sterile conditions, which reduces the exposure of delicate material to harsh disinfectants. Thus, the aseptic culture system of *D. officinale* should be built around explant-specific sterilization, strict donor material selection, and careful handling during inoculation (Sukmadjaja and Widhiastuti, 2019).

2.3 Optimization of culture media and nutritional conditions for *Dendrobium officinale*

Optimization of culture media and nutritional conditions is the core of rapid propagation in *D. officinale*, because different developmental stages require different balances of mineral salts, plant growth regulators, carbon sources, activated carbon, and natural organic additives (Mamun et al., 2018). Across available studies, MS and 1/2 MS are the main basal media, but the optimal formulation varies with explant type and culture objective rather than

following a single universal recipe (Silva et al., 2017). For example, 1/2 MS without phytohormones was reported as optimal for aseptic seed germination, whereas protocorm induction and proliferation were improved on MS supplemented with 1.0 mg/L 6-BA, 1.0 mg/L NAA, and 1.0 mg/L KT (Mehbub et al., 2022). In another seed-based system, the most suitable germination medium was 1/2 MS+0.2 mg/L NAA+100 g/L potato+25 g/L sucrose, while the most suitable rooting and seedling medium was 1/2 MS+0.2 mg/L NAA+100 g/L banana+25 g/L sucrose+0.5 g/L activated carbon (Mamun et al., 2018).

For stem- and node-derived explants, cytokinin-auxin combinations are central to shoot induction and multiplication. In *D. officinale*, effective formulations include MS+1.0 mg/L 6-BA+0.5 mg/L NAA+2 g/L activated carbon for budding stem induction, MS+0.5 mg/L 6-BA+0.8 mg/L 2,4-D+2 g/L activated carbon for proliferation, and 1/2 MS+1.5 mg/L 6-BA+0.5 mg/L NAA for both bud induction and multiple-shoot culture (Mamun et al., 2018). Other studies likewise reported that MS+2.0 mg/L 6-BA+0.1 mg/L NAA gave the highest cluster-shoot proliferation, and that coconut milk, coconut juice, potato extract, banana extract, and activated carbon could further promote PLB propagation, seedling growth, or rooting (Silva et al., 2017; Nguyen et al., 2022). Rooting media generally shift toward reduced salt concentration and stronger auxin support, such as 1/2 MS+2.5 mg/L NAA+15% potato juice+2 g/L activated carbon, MS+0.5 mg/L IBA+0.5 mg/L NAA, or 1/2 MS+0.2 mg/L IBA+0.5% activated carbon+40% potato extract (Mehbub et al., 2022). Rapid propagation of *D. officinale* depends on a stage-specific medium optimization strategy rather than a single fixed medium, and this staged regulation is the basis for high regeneration efficiency and subsequent strong seedling formation.

3 Protocorm Induction and Proliferation Culture

3.1 Conditions for protocorm induction

Protocorm-like body induction in *Dendrobium officinale* depends first on a suitable basal medium and a balanced cytokinin-auxin combination. A targeted study using plumules as explants identified 1/2 MS supplemented with 2% sucrose, 10% banana puree, pH 6.0, plus 6-BA 0.3 mg/L and NAA 0.2 mg/L as the optimal induction condition for PLBs (Fan et al., 2016). More broadly in *Dendrobium* orchids, auxins and cytokinins are the growth regulators most commonly used for PLB induction, and PLBs themselves are considered the most responsive explant type for propagation systems (Arli et al., 2023). Comparative orchid evidence also shows that induction response varies strongly by genotype and regulator type: thidiazuron can be highly effective in some *Dendrobium* systems, while 2iP, meta-topolins, or BA-NAA combinations outperform alternatives in others, so induction formulas should be treated as species- and material-specific rather than universal (Figure 1) (Cardoso et al., 2020).

Induction efficiency is also shaped by explant condition and culture environment after inoculation. In *D. officinale*, light-yellow, loose, and plump PLBs proliferated faster than compact or physiologically aged material, indicating that early visual screening of PLB quality improves subsequent culture performance (Fan et al., 2016). Thin Cell Layer approaches in *Dendrobium* have been reported to outperform larger conventional explants because thinner tissues improve contact with the medium and diffusion into the explant, which is relevant when designing highly responsive induction systems (Arli et al., 2023). At the developmental level, PLBs in *D. officinale* initially show embryoid-like morphology and later shift toward organogenesis, with most protocorms beginning germination and differentiation after about 35 days, so induction protocols should align subculture timing with this transition (Tang et al., 2024).

3.2 Subculture proliferation technology

After induction, subculture proliferation should prioritize physiological uniformity, inoculum density, and transfer interval. In *D. officinale*, inoculating ten PLBs with similar physiological status as one group gave better proliferation performance, and the most significant biomass gain occurred after 45 days of culture, when the proliferation rate reached 1008% (Fan et al., 2016). The same study found that laminated culture favored the upper layer for proliferation and weight gain, and also helped rejuvenate PLBs, suggesting that spatial arrangement in the culture vessel can affect oxygen, light, and nutrient access during repeated passage (Fan et al., 2016). These findings support a practical subculture strategy of selecting vigorous, light-colored PLBs, standardizing inoculum size, and transferring at roughly 45-day intervals before visible decline in vigor (Fan et al., 2016).

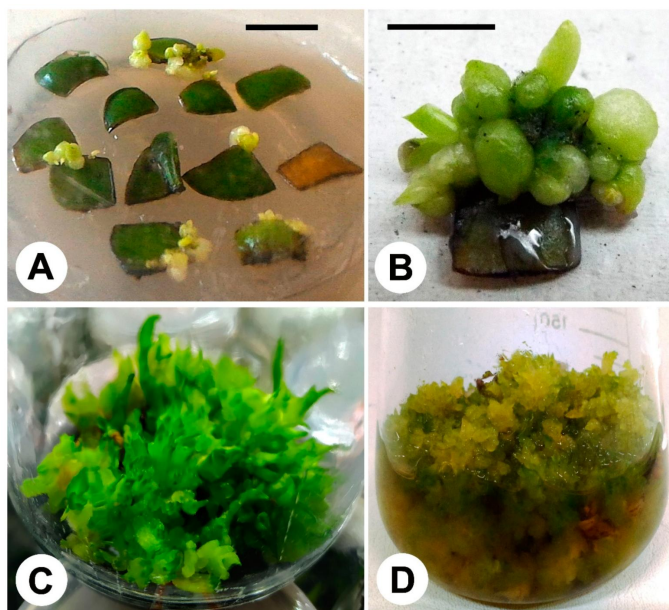


Figure 1 Induction, proliferation and regeneration of protocorm-like bodies in *Dendrobium* and *Phalaenopsis* orchids (Adopted from Cardoso et al., 2020)

Image caption: Protocorm-like bodies (PLBs)-directly induced from leaf segments of *Phalaenopsis* hybrid '501' (A) obtained from young in vitro shoots from inflorescence nodal segments and details of secondary PLBs (B) obtained in New Dogashima Medium (NDM) culture medium. Proliferation of PLBs in agar (C) and liquid (D) MS½ culture medium of *Dendrobium* 'Hybrid 3'. Bars=1 cm. Unpublished photos of Cesar A. Zanello (A,B) and Jean C. Cardoso (C,D) (Adopted from Cardoso et al., 2020)

Hormone and additive optimization remains central during proliferation culture. In a recent *D. officinale* study, 75 µM melatonin accelerated the appearance of white reticulated structures on PLB surfaces and was more conducive to PLB proliferation than the untreated control, whereas 100 µM inhibited adventitious bud differentiation (Tang et al., 2024). Cross-orchid evidence supports the need for species-specific tuning: 2.5 mg/L NAA or 0.5~2.5 mg/L BAP increased PLB percentage and fresh weight in *Dendrobium* sp., while BA with IBA or TDZ plus coconut water improved PLB proliferation and plantlet regeneration in other orchids (Hussien et al., 2024). Modified basal media can also raise PLB biomass without relying heavily on growth regulators; in *Dendrobium Sabin Blue*, a revised half-MS formula increased PLB dry mass by 23% and was proposed to reduce both production cost and the likelihood of somaclonal variation (Chin et al., 2021). Emerging approaches such as liquid shaking culture, temporary immersion systems, and oxygen nanobubbles also appear promising for improving proliferation efficiency, though direct validation in *D. officinale* remains limited (Cardoso et al., 2020; Mawardi et al., 2024).

3.3 Control of browning and vitrification

Browning is one of the main barriers to successful PLB culture because oxidation of phenolic compounds reduces regeneration capacity, suppresses growth, and can cause tissue necrosis or death (Permadi et al., 2024; Kuluev, 2020). Reviews across plant tissue culture agree on several core controls: presoaking explants in antioxidant solutions, incorporating antioxidants such as PVP or ascorbic acid into the medium, using activated charcoal, shortening subculture intervals, and imposing an initial dark treatment (Amente and Chimdessa, 2021; Permadi et al., 2024). Mechanistic evidence shows that browning is closely linked to PPO, POD, and PAL activity; vitamin C, citric acid, activated charcoal, and PVP can all suppress browning to different degrees, with vitamin C performing best in one mechanistic study by reducing PPO and POD activity and lowering total polyphenols (Xu et al., 2023).

Activated charcoal is especially relevant in orchid culture because it adsorbs inhibitory compounds, toxic metabolites, phenolic exudates, and brown exudate accumulation, although it can also adsorb vitamins and plant growth regulators, so its concentration must be optimized rather than added indiscriminately. Additional evidence from recalcitrant species shows that ascorbic acid can outperform other antioxidants in some systems, while activated charcoal at 150~200 mg/L can be better for culture establishment and shoot proliferation in others,

reinforcing that anti-browning recipes remain species-dependent (Guntur et al., 2019; Jakhar et al., 2019). Direct vitrification evidence is sparse in the supplied *D. officinale* corpus, but practical control still follows the same logic as browning prevention: avoid prolonged exposure to excessively high cytokinin levels, maintain moderate inoculum density, and use timely subculture to prevent water-soaked, hyperhydric tissues during rapid proliferation (Fan et al., 2016; Tang et al., 2024). Overall, effective PLB culture in *D. officinale* requires coupling high-proliferation conditions with antioxidant management and disciplined passage schedules, so that rapid multiplication does not come at the cost of tissue quality.

4 Multiple Shoot Induction and Strong Seedling Cultivation

4.1 Regulation of multiple shoot differentiation

The regulation of multiple shoot differentiation in *Dendrobium officinale* depends primarily on the coordinated adjustment of basal medium, cytokinin-auxin balance, and the developmental state of the cultured material. Across *Dendrobium* systems, shoot formation is mainly driven by plant growth regulator composition rather than by any single additive alone, and successful regeneration generally requires species-specific optimization (Pasternak and Steinmacher, 2024; Erkoyuncu, 2026). In related *Dendrobium* species, BA or BAP combined with low to moderate NAA repeatedly promoted shoot proliferation, including MS+1.0 mg/L BA+1.0 mg/L NAA in *D. moniliforme* with a 6.1-fold cluster-shoot proliferation coefficient, MS+3.0 mg/L BAP+1.0 mg/L NAA in *Dendrobium* ‘Red Bull’ with 7.66 shoots per explant, and MS+0.5 mg/L BAP+0.5 mg/L NAA in *D. chryseum* with 5.8 shoots per explant (Mamun et al., 2018; Pathak et al., 2022; Liu et al., 2023). These findings are consistent with broader orchid evidence that cytokinin-dominant media promote shoot induction, while excess auxin tends to redirect development toward callus or rooting rather than repeated shoot differentiation (Kaladharan et al., 2024).

For protocorm-like body and protocorm-derived cultures, multiple shoot differentiation is also strongly influenced by organic supplements and activated charcoal. In *D. crumenatum*, MS+15% coconut water produced 96.0% shooting and 9.5 shoots per explant, while activated charcoal further improved leaf and root development, showing that shoot induction and seedling quality can be uncoupled and optimized sequentially (Klaosheed et al., 2021). Similar responses were reported in *D. thyrsiflorum*, where 0.4 mg/L BA+0.4 mg/L kinetin gave the highest multiplication rate of 4.53 times, and in *D. heyneanum*, where 1.0 mg/L kinetin produced the highest protocorm-derived micropropagation frequency of 90.20% (Figure 2) (Cuc et al., 2022; Kaladharan et al., 2024). Additional evidence from general orchid tissue culture indicates that light quality modifies shoot proliferation by altering sugar accumulation, chlorophyll synthesis, and antioxidant activity, but the response is species-specific, so light should be treated as a secondary regulatory factor after the hormone regime is fixed (Mehbub et al., 2022; Feng et al., 2025).

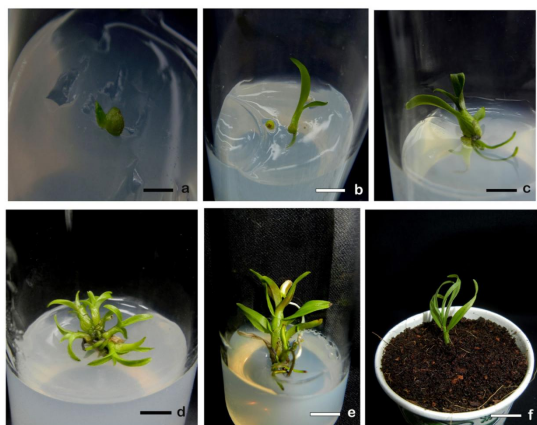


Figure 2 Micropropagation of *Dendrobium heyneanum* Lindl. from protocorms (Adopted from Kaladharan et al., 2024)
Image caption: a) Protocorm (Stage IV); b) Seedling formation from protocorms; c) Shoot with developing pseudobulb and roots; d) Multiple shoot bud formation; e) Elongation of Pseudobulb and roots; f) Hardened plantlet (Adopted from Kaladharan et al., 2024)

4.2 Key measures for strong seedling cultivation

Strong seedling cultivation begins after shoot differentiation and requires synchronized improvement of rooting, leaf expansion, photosynthetic competence, and ex vitro adaptability. In *Dendrobium* orchids, the most common pattern is to shift from multiplication media to lower-salt or auxin-enriched rooting media, often with activated charcoal or organic additives to improve root number and root elongation. In *D. moniliforme*, MS with 0.5 mg/L NAA+0.5 mg/L IBA gave the highest rooting rate, while in *D. chryseum* 2 mg/L IBA gave the highest root number and root length and 2 mg/L NAA performed poorly, indicating that auxin type and concentration affect root quality as much as root induction itself (Liu et al., 2023; Pathak et al., 2022). In *Dendrobium* sp. derived from PLBs, 2.5 mg/L BAP+5.0 mg/L NAA+0.5 mg/L kinetin produced 6.17 roots and 5.10 cm root length, whereas a lower-NAA combination favored shoot regeneration, reinforcing the need for stage-specific rather than one-step media design.

Hardening and transplant preparation are equally important for producing strong seedlings because in vitro plantlets must shift from high humidity and photomixotrophic growth to autotrophic growth under greenhouse conditions. Reviews of *Dendrobium* acclimatization show that gradual reduction of humidity and staged increase in light improve stomatal control, root function, and survival after transfer (Silva et al., 2017). Substrate choice also has a large effect: equal-volume pine bark, turfy soil, and peanut shells performed best for *D. moniliforme*, sterilized coconut husk supported 95% survival in *D. crumenatum*, tree fern produced 94.8% survival in *D. thyrsiflorum*, and chopped coconut husk+brick+charcoal+cocopeat gave the best vegetative hardening response in *D. nobile* plantlets (Klaocheed et al., 2021; Cuc et al., 2022; Liu et al., 2023; Khilari et al., 2023). Even with optimized protocols, survival remains variable across species, ranging from 52.73% in *D. heyneanum* to 92% in *Dendrobium* ‘Red Bull’ and 87% in *D. heterocarpum*, so strong seedling cultivation should be judged by both vigor and post-transplant stability rather than by in vitro growth alone (Mamun et al., 2018; Longchar and Deb, 2022; Kaladharan et al., 2024).

4.3 Quality evaluation of tissue-cultured seedlings

Quality evaluation of tissue-cultured seedlings should integrate morphology, rooting status, physiological performance, acclimatization outcome, and genetic or phenotypic stability. Morphological indicators remain the most practical primary screen, including shoot height, leaf number, leaf size, root number, root length, and overall plant compactness, because these traits respond predictably to medium and environmental optimization (Khilari et al., 2023; Feng et al., 2025). Physiological quality adds another layer: in orchid seedlings, light quality alters chlorophyll, carotenoids, fresh weight, Rubisco activity, and the transition from C3 toward CAM metabolism, showing that visually similar seedlings can differ substantially in photosynthetic readiness for ex vitro growth. General tissue culture evidence also supports the use of soluble sugars, soluble proteins, and antioxidant enzyme activity as indicators linked to proliferation and seedling physiological status (Feng et al., 2025).

Final seedling quality should also be verified by performance after transplanting, because survival and normal development are the most direct tests of whether in vitro seedlings are truly strong. In *D. crumenatum*, regenerated plantlets showed 95% survival and normal greenhouse development, while *D. nobile* plantlets showed 82.3% survival together with high genetic stability by SCoT and IRAP markers, and *D. heterocarpum* showed 87% transplant survival with 96.89% monomorphism in fidelity testing (Klaocheed et al., 2021; Longchar and Deb, 2022). New image-based grading approaches in orchids further show that top-view and side-view imaging can identify root and leaf defects with F1-scores up to 90.44%, suggesting that future *D. officinale* quality evaluation can combine conventional morphological grading with automated digital inspection (Lin et al., 2025). Taken together, high-quality tissue-cultured seedlings of *D. officinale* should be defined not only by rapid in vitro growth, but by balanced shoot-root development, physiological robustness, high transplant survival, and stable true-to-type performance.

5 Rooting and Pre-Transplant Management

5.1 Optimization of rooting culture medium

The optimization of rooting culture medium in *Dendrobium officinale* should follow a stage-specific, reduced-salt, auxin-regulated strategy rather than a single universal formula. In direct studies on *D. officinale*, half-strength MS

was repeatedly used as the basal medium for rooting, and the best reported formulations included 1/2 MS+2.5 mg/L NAA+15% potato juice+2 g/L activated carbon, which produced a rooting rate of 90.4%-92.8%, and 1/2 MS supplemented with 10% banana puree+1.0 mg/L NAA, which produced stronger roots and more vigorous plantlets. Another *D. officinale* optimization study found that two closely related media, both based on 1/2 MS with mashed potatoes, low BA, low NAA, activated carbon, Huabao No.1, and 3% sucrose, were the best overall for rooting and seedling growth promotion. Evidence from related *D. officinale* propagation systems also shows that banana-containing media are useful in the seedling-root growth stage, reinforcing the value of organic additives during rooting culture (Turnip, 2023).

Auxin type and concentration strongly affect the architecture and later usefulness of the root system, but the optimal regulator is not identical across orchid taxa. In *D. officinale*, invigorating culture showed no major vigor difference across 0~2.5 mg/L exogenous auxin overall, yet IBA at 0.75 mg/L or NAA at 0.5 mg/L improved uniformity and vigor, while NAA at 1.0 mg/L with banana puree improved root performance. In other *Dendrobium* species, NAA often increased root number whereas IBA more often promoted root elongation, although some studies reported the reverse depending on concentration and genotype (Mirani et al., 2017; Tini et al., 2025). For example, *D. aqueum* produced 8.75 roots per shoot on 1/2 MS+IBA 5 mg/L, but the longest roots were obtained with NAA 7 mg/L. By contrast, *Dendrobium* cv. Sonia Earsakul and *Phalaenopsis amabilis* both responded best to IBA at 1 mg/L for root length and rooting percentage, while *D. chrysotoxum* showed stronger rooting under relatively high IBA or NAA combined with low cytokinin levels (Sabastian et al., 2022; Alghanimy and Alamery, 2025). These comparisons indicate that for *D. officinale*, medium screening should prioritize half-strength salts, moderate auxin supply, activated carbon, and organic additives, while avoiding the assumption that more auxin always gives better roots (Seliem et al., 2020; Poniewozik et al., 2021).

5.2 Evaluation of root development quality

The evaluation of root development quality in *D. officinale* should go beyond rooting percentage and include root number, root length, uniformity, thickness, vigor, and the subsequent capacity to support acclimatization. Orchid studies consistently identify in vitro rooting as the stage most directly linked to successful transplantation, because acclimatization performance depends primarily on the roots produced before bottle removal (Mirani et al., 2017; Poniewozik et al., 2021). Accordingly, root quality should be judged as a compound trait. In *D. officinale*, the best media were described not only by rooting rate but also by stronger growth, better vigor, and more uniform seedlings. In *D. anosmum*, activated charcoal-containing medium improved shoot length, leaf number, root number, and root length simultaneously, illustrating that a usable transplantable seedling requires balanced whole-plant quality rather than roots assessed in isolation (Nguyen et al., 2022).

Cross-species evidence helps clarify which root traits are most meaningful for pre-transplant assessment. In *D. nobile*, NAA alone outperformed IBA alone for root number, length, and thickness, but combined NAA+IBA reduced rooting and induced callus at the shoot base, showing that visually abundant rooting is not necessarily desirable if root bases are abnormal (Mirani et al., 2017). In *Paphiopedilum insigne*, 1 mg/dm³ IAA or IBA produced many rooted, good-quality plantlets, and the best later acclimatization followed in vitro rooting treatment plus a suitable substrate, indicating that root quality must be validated by ex vitro performance (Poniewozik et al., 2021). In *Phalaenopsis*, nano-selenium plus 0.5 mg/L NAA increased rooting percentage, root length, and root number and then improved acclimatization, again linking root metrics to transplant readiness (Seliem et al., 2020). Practical evaluation in *D. officinale* should therefore classify high-quality roots as numerous enough for anchorage, sufficiently elongated, evenly distributed, free of basal callus or browning, and associated with vigorous leaves and stems, because these combined features are the best predictors of survival after transfer (Mirani et al., 2017; De Stefano et al., 2022).

5.3 Grading of seedlings before bottle removal

Seedlings should be graded before bottle removal because pre-transplant selection improves the consistency of acclimatization management and reduces losses from weak or non-uniform plantlets. The acclimatization literature treats transfer from bottle to external substrate as a critical stage and an important indicator of tissue

culture success, so seedlings entering this stage should first be separated by vigor and developmental completeness (Faradilla et al., 2022; Sari et al., 2023; Turnip, 2023). In *D. officinale*, reports describing improved vigor and uniformity under selected rooting treatments imply that uniform seedlings are a legitimate management target before transplanting. Studies on other orchids show that post-culture evaluation commonly records plantlet height, leaf number, root length, plant height, SPAD, NDVI, and related phenotypes during acclimatization, providing a practical basis for pre-bottle grading criteria (Mullin et al., 2022).

A practical grading system for *D. officinale* before bottle removal should therefore distinguish at least three classes: seedlings suitable for immediate transplanting, seedlings needing continued strengthening, and inferior seedlings to be discarded. Seedlings selected for removal should have a well-developed root system and balanced shoot growth, because such plantlets consistently show higher survival in later hardening studies (Poniewozik et al., 2021; Sarmah et al., 2024). Rooted orchid plantlets grown on 1/2 MS and then shifted briefly to sucrose-free medium acclimatized successfully with 96% survival in one study, suggesting that seedlings should ideally be physiologically prepared, not merely rooted, before transfer. Substrate studies further show that survival varies with media and seedling quality, with favorable outcomes reported in brick+charcoal, coconut coir+charcoal, wood sawdust, and husk-charcoal-containing systems (De Stefano et al., 2022; Sari et al., 2023; Turnip, 2023). Emerging orchid work on automated grading using image-based detection of leaf and root defects achieved F1-scores of 84.44% to 90.44%, indicating that future *D. officinale* production could combine conventional morphological grading with digital inspection for more objective seedling selection (Lin et al., 2025).

6 Acclimatization and Transplant Preparation

6.1 Effects of acclimatization on survival rate

Acclimatization directly determines the ex vitro survival of tissue-cultured *Dendrobium* plantlets because seedlings transferred abruptly from culture vessels to greenhouse conditions rapidly desiccate, wilt, and often die. Gradual adaptation to lower relative humidity and higher light improves survival by allowing plantlets to correct in vitro-induced anatomical and physiological abnormalities, including weak stomatal regulation, poor cuticle function, and incomplete autotrophic competence (Silva et al., 2017). Structural evidence from orchids further shows that hardening strengthens the cuticle, epidermis, mesophyll, and vascular tissues, and these changes were associated with 100% survival in one ex vitro hardening study (Manokari et al., 2023). For *D. officinale* specifically, domestication management during the seedling-training period raised survival during hardening to about 95%, compared with a traditional level of 70%, and post-transplant survival after training exceeded 98%.

Survival after acclimatization also depends strongly on seedling vigor, rooting quality, and biological support. *D. officinale* seedlings generally show low survival and growth after transfer from in vitro conditions, but inoculation with *Mycena dendrobii* significantly enhanced both survival and growth by increasing stress tolerance and promoting new root formation. Other *Dendrobium* studies similarly indicate that pre-hardening on sucrose-free medium or prolonged in vitro strengthening improves later survival, with reported values of 96% in *D. aqueum* and 94% in tea after enhanced in vitro hardening (Bag et al., 2019). Across orchid acclimatization trials, suitable substrates can further stabilize survival, with values of 88.33% in cocopeat, 90% in peat-perlite-bark or river-soil mixtures, and 100% in some wood sawdust or coconut coir systems, showing that acclimatization success reflects both environmental transition and substrate matching (Turnip, 2023; Alghanimy and Alamery, 2025; Luthfi et al., 2026).

6.2 In-bottle and ex-bottle acclimatization measures

In-bottle acclimatization should begin before plantlets are removed from culture vessels, because partial hardening under controlled in vitro conditions reduces transplant shock after transfer. General ex vitro establishment research shows that lowering relative humidity and increasing light intensity before bottle removal can partially harden plantlets in vitro (Mahendra et al., 2020). Prolonged culture also improves transplant readiness: extending the strengthening period increased plantlet growth and raised acclimatization survival from 46% to 94% in one micropropagation system (Bag et al., 2019). In *Dendrobium*, transfer to sucrose-free medium before potting has been used as a transitional step to promote autotrophic adjustment, and *D. aqueum* plantlets cultured for several

weeks on sugar-free 1/2 MS subsequently achieved 96% survival after greenhouse transfer. For *D. officinale*, strong in vitro seedlings with better vigor and uniformity were obtained under moderate auxin regulation, which provides a better material basis for later acclimatization.

Ex-bottle acclimatization should then proceed by staged humidity reduction, light regulation, and use of porous, moisture-retentive substrates. Reviews of *Dendrobium* acclimatization recommend greenhouse hardening under fog or mist conditions with progressive reduction of humidity and gradual increase of light using shading management (Silva et al., 2017). Related hardening systems commonly maintain initial relative humidity near 95% with plastic covers or inverted containers, then gradually reduce moisture and nutrient supply as seedlings move to shade-house conditions (Mahendra et al., 2020). Potting mixtures successful in orchids include brick plus charcoal covered with moss and enclosed with polyethylene bags, peat-perlite-bark combinations, cocopeat, husk charcoal, and bark-based media, all of which improve aeration, drainage, and root spread during the transition period (Alghanimy and Alamery, 2025; Luthfi et al., 2026). In *D. officinale*, bark and moss-based transplant substrates were specifically identified as suitable, and one rapid propagation system further combined bark-moss substrate with gibberellin and *Epulorhiza* sp. during transplanting.

6.3 Cleaning and disinfection after bottle removal

After bottle removal, the first operation should be careful cleaning of the root system to remove residual medium, because attached medium can retain excess moisture and increase contamination risk during transplant establishment. General hardening reviews state that regenerated plantlets are usually separated completely from the MS medium and the roots are thoroughly washed before planting (Mahendra et al., 2020). This step is especially important because newly exposed roots and basal tissues are highly vulnerable to soil microorganisms during early acclimatization, and sudden exposure to microbial communities is a major cause of mortality in tissue-cultured plants (Bag et al., 2019). Accordingly, many systems use initially sterilized or disinfected substrates to reduce pathogen pressure during establishment (Bag et al., 2019). In *D. officinale* seedling domestication practice, management after bottle and subsequent field planting are treated as distinct technical links, underscoring the importance of post-bottle cleaning and handling for maintaining high survival.

Disinfection after bottle removal should balance microbial control with avoidance of phytotoxic injury. In orchid systems, chemical disinfestation with commercial bleach has been used for re-cultivation, and one study found no significant growth difference between plantlets transferred in autoclaved vessels and those managed with bleach-based disinfection (Fontes et al., 2017). However, disinfectant concentration is critical, because increasing sodium hypochlorite levels can reduce germination or increase toxicity, while lower concentrations can still control contamination. For orchid explants more broadly, effective sterilization has been reported with 70% ethanol followed by sodium hypochlorite or HgCl₂, but stronger or prolonged treatments can increase mortality (Kajol et al., 2024). Evidence from hydrogen peroxide treatments suggests that H₂O₂ is more effective against fungal than bacterial contamination, so it should not be relied on alone when bacterial pressure is high (Mtd et al., 2026). Therefore, post-bottle preparation of *D. officinale* should combine root washing, removal of damaged tissues, transplanting into clean porous substrate, and cautious use of low-to-moderate disinfection intensity to reduce contamination without compromising seedling viability.

7 Transplant Survival Improvement Techniques

7.1 Selection and formulation of transplant substrates

The transplant substrate is a primary determinant of post-bottle establishment in *Dendrobium officinale* because it controls aeration, water retention, root anchorage, and microbial exposure during the most vulnerable recovery period. Direct *D. officinale* studies identified several effective formulations, including vermiculite:perlite:humus soil at 1:1:5, which was selected as the corresponding transplant medium in an industrialized regeneration system, and bark-based matrices, with pure bark giving the highest survival in one rapid propagation study. Another *D. officinale* protocol recommended a bark-moss base combined with *Epulorhiza* sp., while substrate metabolomics work showed that pine bark, coconut coir, and their 1:1 mixture all support cultivation, but they produce different metabolic outcomes, underscoring that substrate choice affects both survival and seedling quality (Zhang et al., 2024).

Broader orchid evidence supports using porous, moisture-buffering substrates with stable structure during acclimatization. Cocopeat gave the highest survival at 88.33% in one orchid study, coconut coir outperformed a sphagnum-charcoal-bark combination for seedling height and leaf number in another, and wood sawdust or coconut coir produced 100% early survival in a hardening experiment (Silva et al., 2017; Longchar and Deb, 2022; Han et al., 2025). For *Dendrobium* specifically, sterilized coconut husk supported 95% survival in *D. crumenatum*, tree fern supported 94.8% survival in *D. thyrsiflorum*, and *D. nobile* performed best in a mixed substrate of chopped coconut husk, brick, charcoal, and cocopeat. Together, these results suggest that *D. officinale* transplant substrates should prioritize bark- or coir-based materials with good drainage and moderate moisture retention, while local optimization remains necessary because performance differs by genotype and cultivation objective (Rachmawati et al., 2024).

7.2 Temperature, humidity, light, and ventilation management

Environmental management after transplanting should follow a gradual transition rather than an abrupt exposure to ambient conditions. In *Dendrobium*, acclimatization improves survival by progressively lowering relative humidity and increasing light, allowing plantlets to shift from heterotrophic or photomixotrophic growth to autotrophic growth while improving stomatal control and root function (Silva et al., 2017). More general orchid hardening guidance similarly recommends maintaining relative humidity near 95% at first, then gradually reducing moisture and nutrient supply while moving seedlings into shaded conditions, which promotes wax formation, thicker leaves, and more stable water relations (Zhang et al., 2022). In *D. officinale* domestication practice, greenhouse seedling training for about three months produced thicker leaves, darker color, stronger stems, more robust roots, and post-transplant survival above 98%, indicating that controlled environmental transition materially improves transplant success (Rachmawati et al., 2024).

Light and ventilation must be coordinated with seasonal temperature control because excessive heat and stagnant humidity increase stress and disease risk. A *D. officinale* cultivation study reported that plantlets were properly shaded, cooled, and ventilated during summer and autumn, linking these measures to improved growth quality under cultivation (Zhang et al., 2024). Orchid precision-greenhouse research further shows that intelligent environmental management can track growth status and support decisions on greenhouse factors, with recognition accuracy reaching 98.6%, which supports future refinement of temperature, humidity, light, and ventilation control in orchid nurseries. Physiological evidence also indicates that appropriate light and potassium treatments can increase anthocyanin content and that different cultivation modes affect active compound accumulation, so environmental management during recovery should aim not only at survival but also at maintaining medicinal quality (Zhang et al., 2024).

7.3 Disease prevention and management during the seedling recovery period

Disease prevention during the recovery period should begin with sanitation and microbial risk reduction because newly transplanted tissue-cultured seedlings are highly susceptible to pathogen attack. General acclimatization studies note that a major cause of mortality is the rapid exposure of aseptically raised roots to soil microbial communities, especially fungi, and sterilized soil is therefore often used at the beginning of establishment (Rachmawati et al., 2024). Post-bottle handling should include thorough washing to remove residual medium from the roots before planting, since retained medium can favor excess moisture and contamination (Silva et al., 2017). These sanitation steps fit *D. officinale* domestication systems, which explicitly treat post-bottle handling and post-field management as separate technical links within survival-oriented nursery management (Rachmawati et al., 2024).

Biological management also appears important for disease resistance and recovery vigor in *D. officinale*. *Mycena dendrobii* significantly enhanced survival and growth after transplanting, and proteomic analysis indicated that the induced proteins included defense-and stress-response proteins, supporting a disease-preventive role through improved stress tolerance rather than simple growth promotion alone. Other orchid mycorrhizal studies found that fungal symbiosis can raise antioxidant enzyme activity, improve drought and disease resistance, and increase root and leaf growth, biomass, and medicinal polysaccharides, although effects differ by fungal strain and development

stage (Zhang et al., 2020; Zhang et al., 2022). During the recovery period, disease management should therefore combine clean substrate, careful watering, adequate ventilation, and targeted use of beneficial fungi, while avoiding overly complex fungal mixtures because synthetic combinations did not show synergistic effects and sometimes produced offset effects (Wu et al., 2025).

8 Conclusions and Prospects

Tissue culture rapid propagation is the most practical basis for producing high-quality *Dendrobium officinale* seedlings because conventional propagation provides limited offspring and does not support industrial-scale seedling supply, whereas in vitro systems can establish continuous regeneration from germination, protocorm induction, proliferation, rooting, hardening, and transplanting. Direct studies on *D. officinale* have shown that stage-specific optimization of medium composition can support efficient germination, shoot proliferation, rooting, and transplant preparation, although the best formula differs by explant and culture phase. The same body of work indicates that high-quality seedlings are not defined only by multiplication rate, but by uniformity, strong rooting, and later transplant competence, which is why complete propagation systems are more valuable than isolated induction protocols. Broader *Dendrobium* evidence further supports this conclusion by showing that tissue culture is already an established propagation route for the genus and also provides the technical basis for germplasm conservation, clonal fidelity control, synthetic seed, bioreactor, and transformation technologies. In related medicinal *Dendrobium* species, optimized protocols produced about 87% transplant survival with 96.89% monomorphism after transfer, while *D. nobile* showed high genetic stability and 82.3% acclimatization survival, indicating that rapid propagation can support both scale and seedling quality when protocol stability is maintained.

Improving the transplant survival rate of *D. officinale* requires multi-stage coordination because losses arise not from a single step, but from cumulative mismatches among rooting quality, acclimatization intensity, substrate properties, and post-transplant stress response. Reviews of *Dendrobium* acclimatization show that plantlets must be gradually adapted to lower humidity and higher light so they can restore stomatal regulation, root function, and autotrophic growth before field or greenhouse establishment. Direct *D. officinale* studies also show that survival changes markedly with substrate and transplant management, with high-performing systems reported for pure bark, bark-moss bases, and vermiculite:perlite:humus soil mixtures, while broader orchid studies similarly favor porous, moisture-buffering media rather than overly compact substrates. Biological coordination is another important layer, because *Mycena dendrobii* significantly enhanced survival and growth of *D. officinale* seedlings by inducing defense- and stress-response proteins and promoting new root or mycorrhizal formation. Mycorrhizal evidence further shows that fungal effects are development-dependent and strain-specific: some fungi promote germination, others favor biomass, rooting, tillering, or polysaccharide accumulation, while synthetic fungal combinations do not necessarily produce synergistic effects. Therefore, future survival-improvement strategies for *D. officinale* should integrate strong-seedling culture, graded acclimatization, substrate matching, and targeted microbial assistance into one continuous technical chain rather than optimizing each step in isolation.

Standardization and intelligent management appear to be the clearest future directions for *D. officinale* seedling propagation because current orchid tissue-culture research is increasingly focused on medium optimization, endophytic-fungal regulation, and systematic solutions to contamination, browning, and vitrification, yet protocol fragmentation still limits reproducibility across genotypes and production sites. Recent bioreactor work in *Dendrobium* shows that liquid culture, temporary immersion systems, anti-browning additives such as ascorbic acid, and banana-extract-based regeneration media can markedly improve multiplication efficiency and may provide a foundation for standardized commercial systems, although genotype-specific optimization remains necessary. Orchid studies outside *D. officinale* also show that medium formulation depends on cultivation system and that combining optimized media with temporary immersion technology can reduce cost while balancing regeneration efficiency and genetic stability, which is directly relevant to future seedling industrialization. At the management level, intelligent greenhouse technology can already identify orchid growth status with 98.6% recognition accuracy and support environmental control and decision-making, suggesting practical value for digital monitoring of seedling vigor, environmental fluctuations, and transplant recovery. Taken together, the

future of *D. officinale* seedling propagation lies in building standardized, data-driven technical systems that combine rapid propagation, clonal quality control, microbial regulation, bioreactor scaling, and intelligent greenhouse management, so that seedling production becomes more stable, economical, and suitable for large-scale medicinal cultivation.

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Feature Review

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Construction of a Technical System for Rhizome Propagation and High-Quality Seedling Production of *Polygonatum sibiricum*

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Abstract As an important medicinal-and-edible homologous plant, *Polygonatum sibiricum* has long faced challenges such as declining wild resources, germplasm confusion, and insufficient supply of high-quality seedlings, which restrict the large-scale and standardized development of the industry. This paper focuses on rhizome propagation and high-quality seedling production of *P. sibiricum* and systematically constructs a technical system from the biological basis of propagation to large-scale production. First, the biological basis of rhizome regeneration and germination is clarified from the perspectives of rhizome structural characteristics, reserve accumulation, and environmental regulation mechanisms, highlighting the key roles of bud differentiation and carbohydrate reserves in vegetative propagation capacity. Second, multiple propagation pathways, including division propagation, segment propagation, and tissue culture, are systematically summarized, and their differences in propagation efficiency, resource consumption, and industrial applicability are compared. A production model combining rapid multiplication through tissue culture with field division for mother-stock maintenance is proposed. In seedling production, a standardized cultivation system is further constructed from the aspects of site selection, substrate formulation, water-fertilizer management, shading regulation, and green pest and disease control. Finally, a comprehensive quality grading standard integrating morphological indicators, root traits, physiological and biochemical indicators, and stress resistance evaluation is established, while storage and vigor maintenance techniques are incorporated to improve the seedling production chain. This system aims to enhance seedling uniformity and propagation efficiency of *P. sibiricum*, providing theoretical and technical support for rapid multiplication of elite germplasm, standardized production, and sustainable industrial development.

Keywords *Polygonatum sibiricum*; Rhizome propagation; Tissue culture; Seedling quality evaluation; Standardized production

1 Introduction

Polygonatum sibiricum is a widely used traditional medicinal and edible plant in East Asia, and its rhizome has been recorded in classical materia medica and used for food and medicine for centuries. Modern studies show that its rhizomes contain polysaccharides, saponins, flavonoids, alkaloids, phenolic acids, and other bioactive constituents that support broad pharmacological activities, including antioxidant, anti-inflammatory, hypoglycemic, immunomodulatory, antitumor, neuroprotective, and lipid-regulating effects (Pan et al., 2022; El-Hack et al., 2025). Among these constituents, *Polygonatum sibiricum* polysaccharides are regarded as major active substances and have attracted sustained attention for their roles in functional foods, nutraceuticals, and pharmaceutical development (Cui et al., 2018; Yang et al., 2024). In addition to medicinal application, *Polygonatum sibiricum* is recognized in China as a medicinal-and-edible homologous resource and has been developed into teas, beverages, oral liquids, powders, pills, and other health products, indicating a clear extension from traditional herb use to diversified industrial utilization (Li et al., 2025). This expanding scientific basis and product diversification have increased the medicinal, nutritional, and economic value of *Polygonatum sibiricum*, making it an important raw material for the health industry and a promising forest-understory crop (Su et al., 2018; Liao et al., 2023).

Driven by these values, market demand for *Polygonati Rhizoma* has risen substantially in recent years, and artificial cultivation has gained attention because demand has outpaced the supply capacity of natural populations

(Cheng et al., 2023). Evidence from industry and cultivation studies shows that wild resources have been heavily depleted by overexploitation, long-term harvesting pressure, and the long growth cycle required to form harvestable rhizomes (Su et al., 2018). This contradiction is likely to intensify under environmental change, because habitat suitability for *P. sibiricum* is projected to decline in the future and shift geographically, adding further pressure to conservation and germplasm utilization (Zhang et al., 2023). Although cultivated *Polygonatum sibiricum* is now an important source for market supply and can provide more stable production, persistent problems remain, including germplasm confusion, unstable quality, low cultivation technology, and insufficient supply of high-quality seedlings (Pan et al., 2022). Seedling production is a central bottleneck because large-scale cultivation depends on reliable nursery systems, yet seed propagation is constrained by deep morphophysiological dormancy, slow germination, and delayed seedling establishment (Liao et al., 2021). Even under production conditions, harvestable rhizomes generally require 3–4 years through rhizome propagation and 5–6 years from seed propagation, which limits rapid expansion and raises the importance of efficient vegetative propagation and early seedling management.

At the same time, the transition from wild collection to cultivation has created higher requirements for seedling standardization and quality consistency. Comparative metabolomic studies indicate that cultivated and wild-grown *P. sibiricum* share many important phytochemicals, but they can also differ in specific compounds and abundance profiles, which means that propagation materials and cultivation practices can directly affect product quality (Pan et al., 2022). Encouragingly, artificially cultivated rhizomes with multiple buds show phytochemical profiles broadly comparable to wild types and contain several therapeutically relevant compounds, supporting the feasibility of cultivation-based substitution if propagation systems are well designed (Cheng et al., 2023). Research on rhizosphere adaptation further suggests that yield and quality are strongly influenced by site conditions such as soil pH and associated beneficial or pathogenic microorganisms, highlighting that high-quality seedling production must be integrated with suitable ecological and agronomic management (Shi et al., 2024). Studies on distribution, phenotype, and common-garden performance likewise show marked variation among *Polygonatum* materials in yield, quality traits, and environmental adaptation, reinforcing the need for standardized propagation pathways, germplasm selection, and nursery evaluation indicators before field expansion (Liao et al., 2023). Therefore, establishing a technical system for rhizome propagation is not only a matter of multiplying planting material, but also a prerequisite for preserving elite germplasm, stabilizing medicinal quality, and supporting scalable industrial production.

This study will explore the construction pathway of a technical system for rhizome propagation and high-quality seedling production of *Polygonatum sibiricum*, with a focus on key issues such as selection of propagation materials, improvement of propagation efficiency, control of seedling uniformity, and subsequent cultivation adaptability. This system is expected to provide technical support for the rapid multiplication of elite germplasm, reduce dependence on wild resources, and promote the large-scale production of standardized seedlings. Meanwhile, this study is of great significance for improving the consistency of raw medicinal materials of *Polygonatum sibiricum*, ensuring the stable supply of medicinal-and-edible homologous products, and promoting industrial modernization. From a broader perspective, the construction of this technical system will help achieve the coordinated integration of germplasm conservation, standardized production, and efficient resource utilization, thereby providing a theoretical and practical basis for the sustainable and high-quality development of the *Polygonatum sibiricum* industry.

2 Biological Basis of Rhizome Propagation

2.1 Structural characteristics

The underground organ of *P. sibiricum* is a perennial rhizome that extends by producing one new segment each year, forming a distinct “annual rhizome” pattern that provides the structural basis for clonal propagation. Within *P. sibiricum*, annual rhizomes show at least two recognizable morphological types, including a thick-ended “Jitou-type” and an atypical type without obvious thickening, indicating that rhizome architecture is variable even within the species (Hu et al., 2022). Artificially cultivated materials with multiple rhizome buds have also been

identified and compared with wild plants bearing single or multiple buds, showing that bud number is a relevant structural trait in cultivation and germplasm evaluation (Cheng et al., 2023). Across *Polygonatum*, rhizomes retain typical monocotyledonous anatomical organization, but species-level differences occur in vascular bundle type, and in *Polygonatum* rhizomes collateral, amphivasal, and incomplete amphivasal bundles have all been observed (Cantürk and Özhatay, 2021).

Bud formation on the rhizome is not random, but follows a recognizable developmental pattern associated with annual segment formation and latent bud differentiation. In some *Polygonatum* germplasm, annual rhizomes carry two developed latent buds with unequal vigor, one long and stout and the other short and thin, suggesting a built-in hierarchy among buds that can influence which propagules dominate subsequent growth. By contrast, other rhizome types show short, small, or underdeveloped latent buds, indicating that the regenerative potential of different rhizome segments is partly constrained by bud morphology itself (Hu et al., 2022). Developmental anatomy further shows that, during seed-derived establishment, the hypocotyl swells into a newborn rhizome and becomes the site on which the germ and radicle differentiate, demonstrating that rhizome formation is an early organizing event in the plant's life cycle rather than merely a later storage response. Because the first rhizome links emerging organs with nutrient transfer tissues, its structural integrity and bud-bearing capacity directly determine the material basis for subsequent vegetative multiplication.

2.2 Physiological basis

The regenerative capacity of *P. sibiricum* rhizomes depends on their strong reserve accumulation function. Histochemical evidence shows that polysaccharides are localized in mucilage cells of *Polygonatum* rhizomes, while saponins and volatile oils are mainly distributed in the ground tissue, indicating spatial differentiation of storage and defense-related metabolites within the underground stem. Chemical studies further confirm that the rhizome is rich in carbohydrates and polysaccharides, which are major bioactive and nutritional components of the species. Detailed carbohydrate profiling suggests that *P. sibiricum* rhizome does not rely mainly on starch; instead, fructo-oligosaccharides are a major component and account for about 28.95% of the rhizome, with higher-degree polymers particularly abundant. This reserve composition implies that nutrient storage in the rhizome is based largely on soluble and structurally diverse carbohydrate pools that can support both medicinal quality formation and regrowth after segment division.

Age-related comparisons show that young rhizomes already possess substantial reserve value: the polysaccharide extraction rate of young rhizomes reached 33.88%, close to 45.08% in mature rhizomes, and their main polysaccharide fractions showed similar structural features and biological activity. This finding supports the physiological feasibility of using younger rhizome material as propagation stock, because immature segments are not nutritionally empty organs but already contain considerable carbohydrate reserves. Regeneration also depends on efficient reserve mobilization. During germination, the haustorium absorbs nutrients from the endosperm and transfers them through the cotyledonary structure to the newborn rhizome, providing the material basis for differentiation of the germ and radicle. At the molecular level, dormancy release and seedling establishment involve broad changes in hormone metabolism genes, cell-wall-related genes, endosperm weakening, carbohydrate metabolism, and amino acid synthesis, indicating that regenerative growth arises from coordinated reserve remobilization rather than passive bud swelling alone (Liao et al., 2021; Gou et al., 2026).

2.3 Environmental regulation

Environmental regulation is a decisive factor controlling rhizome germination and early growth in *P. sibiricum*. Under natural conditions, seeds are dispersed in autumn, produce radicle and corm structures only after warm conditions in the following growing season, and often do not complete shoot emergence until after a second winter, reflecting deep epicotyl morphophysiological dormancy (Liao et al., 2021). Controlled sequential temperature treatment markedly shortens this cycle: warm stratification at 25 °C for 4~6 weeks promotes radicle extrusion and cormlet formation, followed by cold stratification at 4 °C for 8 weeks, after which return to 25 °C induces seedling emergence. Additional studies similarly found that 0 °C sand storage for 120 days favors germination, and recent optimization experiments predicted about 89.31% germination under a combined regime

of GA₃, about 4.90 °C, and a 1:1 sand-to-Huangjiang-slag substrate ratio (Gou et al., 2026). Temperature, light, water, and nitrogen are recognized as core environmental signals controlling dormancy and germination through interaction with ABA and GA pathways.

Light and moisture conditions further modify germination performance. Dark treatment increased *P. sibiricum* seed germination relative to illumination, and soaking seeds in 35~40 °C lukewarm water for 18~24 h raised germination to 93.33%, indicating that both light exclusion and improved imbibition can facilitate dormancy release. Hormonal regulation mediates much of this environmental response: cold stratification for more than 70 days significantly alleviated dormancy, ABA remained high during dormancy, and exogenous GA₃ and 2-coumarate promoted germination while 6-BA and GA₃ enhanced corm growth. Transcriptomic and metabolomic evidence likewise indicates that ABA helps maintain dormancy whereas GA₃, JA, and IAA rise during seed coat penetration and promote germination-associated processes (Gou et al., 2026). After emergence, rhizome growth and quality remain sensitive to habitat conditions: *P. sibiricum* is commonly distributed in moist and cool forest or shrub environments, artificial and wild plants examined in Fujian grew under high precipitation, 82% relative humidity, and moderate annual temperature (Cheng et al., 2023), rhizome fresh weight and polysaccharide content decline with soil acidification, and poor performance is associated with rhizospheric pathogens such as *Fusarium* (Shi et al., 2024), including field-reported root rot beginning in the rhizome and reaching a disease index of 70% in Gansu plantations.

3 Rhizome Propagation Technology System

3.1 Division and whole-rhizome propagation methods and their characteristics

Rhizome propagation is a core propagation pathway for *Polygonatum sibiricum* because seed propagation is constrained by a long juvenile cycle and deep epicotyl dormancy, whereas rhizome-derived plants reach harvestable rhizomes in about 3~4 years rather than 5~6 years from seed (Meucci et al., 2024). Natural rhizome propagation is still inherently slow, which is why conventional field multiplication based on whole rhizomes or larger bud-bearing pieces remains reliable but inefficient for rapid scale-up (Tejera-Nieves and Walker, 2023). In *Polygonatum* germplasm, some cultivars form one or multiple branch buds adjacent to the renewal bud in the next growth season, and multi-bud rhizome types usually have larger rhizome segments and are more favored in cultivation, which gives whole-rhizome propagation an advantage for maintaining strong early vigor and varietal traits (Lubbe et al., 2023). Comparative metabolomic work also found that artificially cultivated plants with multiple rhizome buds had phytochemical profiles generally comparable to wild types, supporting the production value of clonal rhizome-based multiplication (Figure 1) (Cheng et al., 2023).

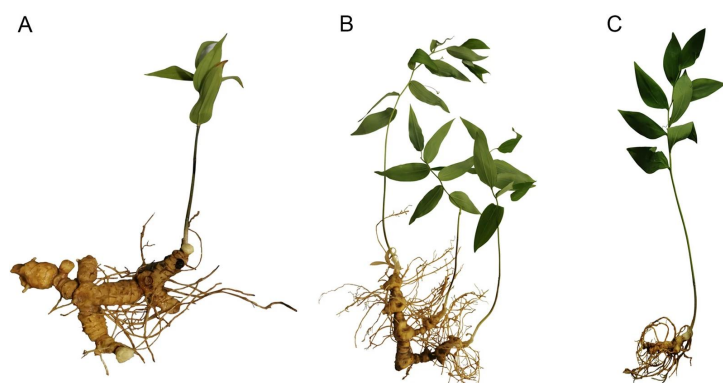


Figure 1 The photos of the rhizomes of *P. sibiricum* from the artificial cultivation with multiple rhizome buds (A), the wild-type with multiple rhizome buds (B), and the wild-type with single rhizome bud (C)

The main advantage of whole-rhizome propagation is that it preserves a more complete storage organ and therefore tends to maintain stronger nutrient reserves, bud viability, and transplant buffering capacity, while the main disadvantage is the low multiplication coefficient and the sacrifice of more commercial rhizome material per seedling (Meucci et al., 2024). Division propagation increases the propagation coefficient by cutting the rhizome into bud-bearing units, but its performance depends on retaining viable buds and sufficient storage tissue around

each bud (Stephen et al., 2023). Annual rhizome segments in *Polygonatum* differ in bud morphology, and some latent buds are long and stout while others are short, thin, or underdeveloped, so division should preferentially use robust, clearly differentiated buds rather than inconspicuous or weak bud positions. In practice, whole-rhizome or large-division propagation is more suitable for conserving elite germplasm and establishing mother-stock nurseries, whereas smaller division is more appropriate when the objective is to expand planting material rapidly under controlled nursery management.

3.2 Technical key points and optimization of segment and bud-section propagation

The technical core of segment and bud-section propagation is balancing multiplication rate against bud survival and reserve sufficiency. Structural studies show that *Polygonatum* annual rhizomes contain latent buds with unequal developmental strength, and underdeveloped buds are less suitable as independent propagules (Stephen et al., 2023). Physiological studies further show that the rhizome is a major storage organ rich in polysaccharides and total sugars, while young rhizomes already retain substantial polysaccharide levels close to mature rhizomes, which supports the use of appropriately developed young bud-bearing segments as nursery material rather than discarding them (Khan et al., 2025). This means optimized segmentation should retain both one effective bud and enough surrounding rhizome tissue to support early sprouting, rooting, and post-cut recovery.

Environmental and hormonal regulation strongly affects the success of these smaller propagules. Cold stratification at 4 °C for more than 70 days alleviated dormancy in *P. sibiricum*, and exogenous GA3 promoted germination and corm growth, indicating that temperature conditioning and hormone-assisted bud activation can improve the performance of bud sections after cutting (Khan et al., 2025). More broadly, temperature, water, and light act as key signals controlling dormancy release and germination, and dark conditions, low-temperature sand treatment, and warm-water soaking improved *P. sibiricum* germination in seed studies, which supports the same general principle of pre-plant conditioning for vegetative propagules (Lubbe et al., 2023). Because rhizome performance also declines under unfavorable soil biotic conditions, nursery substrates for bud sections should avoid acidic, pathogen-prone environments; in *Polygonatum*, rhizome fresh weight and polysaccharide content were highest at soil pH 7.48-7.95, while poor yield and quality were associated with pathogens such as *Fusarium* in the rhizosphere (Meucci et al., 2024). Accordingly, optimization of segment and bud-section propagation should integrate propagule grading, sanitation, temperature pretreatment, and a clean substrate environment rather than relying on cutting strategy alone.

3.3 Application of tissue culture rapid propagation in large-scale production

Tissue culture is the most scalable propagation route for *P. sibiricum* because it can generate large numbers of genetically uniform, pathogen-reduced propagules in limited space and throughout the year (Khan et al., 2025; Nawaz et al., 2025). Existing *P. sibiricum* patents and experimental studies already show that buds from current-year subterranean stems and tuber parts with hidden bud points can be used for cluster-bud induction, callus culture, multiplication, rooting, seedling hardening, and transplanting, with the explicit aim of rapid reproduction and large-area industrialized planting (Stephen et al., 2023). An earlier asexual line system for *P. sibiricum* identified suitable media for growing-point development, adventitious bud differentiation, and rooting, and field planting showed that tube seedlings grew well and increased rhizome yield by 50%. Another rapid-propagation method based on disinfected budded root tubers used cyclic enrichment and bud proliferation for 3–6 generations and was described as applicable to scale production (Marsh et al., 2023).

The key technical challenge is not whether micropropagation works, but whether it can be made simple, reproducible, and economical at production scale. Reviews of commercial micropropagation identify contamination, low multiplication rate, media cost, and acclimatization losses as the main barriers, and they emphasize the need for protocols with high reproducibility and high ex vitro survival (Nawaz et al., 2025). Practical scaling strategies include liquid culture systems, simplified media, and conservation-compatible slow-growth storage systems that reduce labor and maintain viable propagules between production cycles. Related rhizomatous species also show that cold-stored encapsulated microrhizomes or slow-growth cultures can survive for months to a year, supporting the feasibility of integrating storage, transport, and staged nursery supply into a

tissue-culture-based production chain (Khan et al., 2025). For large-scale *P. sibiricum* production, tissue culture is therefore best positioned as the rapid multiplication platform, while field rhizome division remains the lower-cost method for mother-stock maintenance and local nursery expansion.

4 Rhizome Formation and Harvest Standards

4.1 Selection of mother plants and quality control of rhizome sources

Mother-plant selection for *Polygonatum sibiricum* rhizome propagation should prioritize stable agronomic performance, strong rhizome development, and high levels of functional constituents, because *Polygonatum* materials differ substantially in yield, polysaccharide content, saponin content, and related morphological traits under common-garden conditions. Provenance also matters, since the formation of plant genetic traits differs among source populations and is affected by climatic conditions at the place of origin. In practical selection, stem diameter can serve as an auxiliary field indicator because it is positively correlated with yield and has been proposed as an indicator for harvest-oriented variety screening. Mother plants should therefore be chosen from vigorous, disease-free stands with consistent aboveground growth and strong rhizome expansion, while avoiding mixed or poorly adapted germplasm that can reduce uniformity in subsequent seedling production (Figure 2) (Liao et al., 2023).

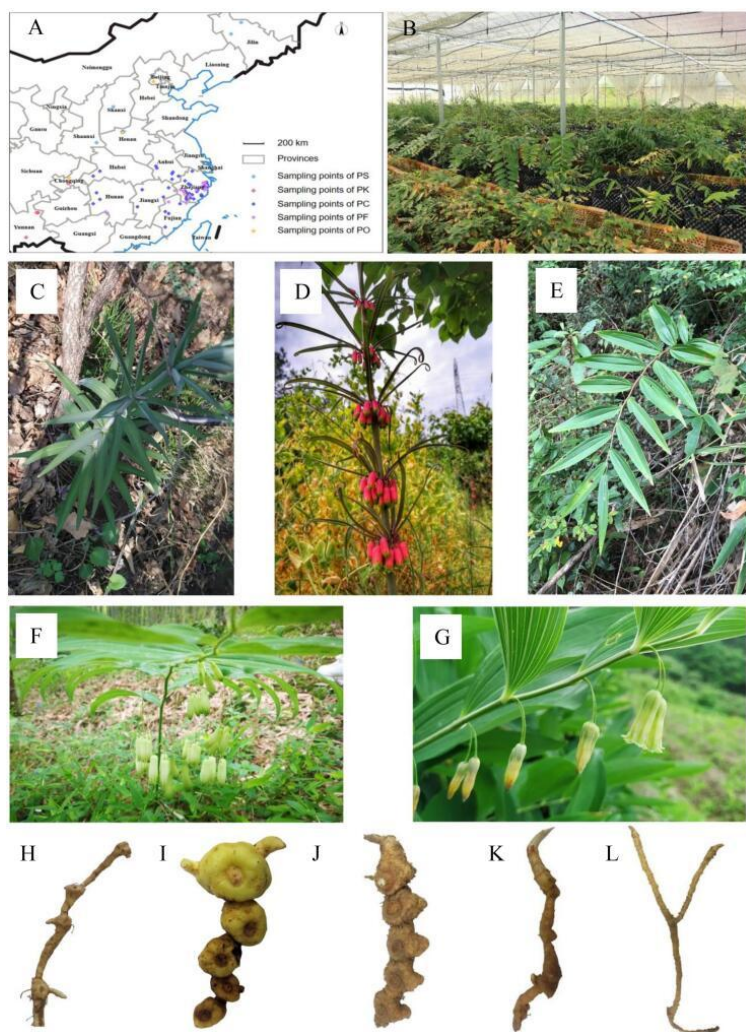


Figure 2 The sampling distribution and morphology of five species from *Polygonatum* genus (Adopted from Liao et al., 2023)
Image caption: (A) Sampling distribution of 5 different species from *Polygonatum* genus populations in map of China (partial); (B) The common garden in Zhejiang, China; (C) The aerial part of *Polygonatum sibiricum* Red. (PS); (D) The aerial part of *P. kingianum* Coll. et Hemsl. (PK); (E) The aerial part of *P. cyrtoneura* Hua (PC); (F) The aerial part of *P. filipes* Merr. (PF); (G) The aerial part of *P. odoratum* (Mill.) Druce (PO); (H) The rhizome morphology of PS; (I) The rhizome morphology of PK; (J) The rhizome morphology of PC; (K) The rhizome morphology of PF; (L) The rhizome morphology of PO (Adopted from Liao et al., 2023)

Quality control of rhizome sources should combine morphological authentication with chemical consistency assessment. Rhizomes of *P. sibiricum* show recognizable type differences, and comparative evaluation of Polygonati rhizoma has shown that different rhizome forms have different comprehensive quality, with BJPR and JTPR performing better in nutritional and medicinal evaluation (Hu et al., 2022). At the same time, cultivated *P. sibiricum* can provide a stable supply, but its phytochemical profile is not identical to that of wild material, with 33 phytochemicals differing significantly between cultivated and wild-grown rhizomes (Pan et al., 2022). Even so, artificially cultivated rhizomes with multiple buds show an overall phytochemical profile comparable to wild types and contain important therapeutic compounds, supporting their use as propagation sources when identity and quality are verified (Cheng et al., 2023). For source control, rapid screening tools such as near-infrared spectroscopy combined with chemometrics are also valuable because they provide efficient assessment of polysaccharide and saponin levels and are considered useful for preliminary quality evaluation and raw-material grading (Liao et al., 2023).

4.2 Determination of growth duration and harvest maturity of rhizomes

The determination of rhizome harvest timing should be based on the coordinated evaluation of cultivation age and seasonal stage, because both factors directly affect the accumulation of functional components. Evidence from long-term research on Polygonatum rhizomes shows that quality-related metabolites increase significantly during early-to-middle growth years and then decline after peak accumulation; in a five-year growth cycle, functional components increased from years one to four and decreased from years four to five, with the most active accumulation occurring between years three and four. The four-year-old rhizomes showed the most superior overall quality because they contained higher levels of functional components and more newly formed metabolites (Pu et al., 2024). Although these data come from *P. cyrtoneura*, they provide a strong reference for *P. sibiricum* harvest-standard setting within Polygonatum, indicating that harvest maturity should not be judged solely by rhizome size, but by the balance between biomass formation and metabolite accumulation.

Season also changes rhizome quality, so harvest maturity standards should distinguish between spring and autumn products rather than treating them as equivalent. Comparative seasonal analysis showed that rhizomes harvested in spring and autumn had unequal quality because of significant differences in functional composition; spring rhizomes contained more flavonoids, alkaloids, and phenolic acids, whereas autumn rhizomes contained more steroids (Pu et al., 2024). For *P. sibiricum*, maturity standards should also recognize that young rhizomes are not devoid of value, since their polysaccharide extraction rate reached $(33.88 \pm 1.95)\%$, compared with $(45.08 \pm 1.92)\%$ in mature rhizomes, and their polysaccharide structure and biological activity were broadly similar to those of mature rhizomes. This means mature rhizomes remain the main target for medicinal harvesting, but younger rhizome fractions can still be classified for propagation or comprehensive utilization rather than discarded as waste.

4.3 Grading indicators and commercialization standard system for rhizomes

A commercialization standard system for *P. sibiricum* rhizomes should integrate morphological, agronomic, and chemical indicators into a graded evaluation framework. Morphological indicators should include rhizome type, fullness, segmentation regularity, bud number, diameter, and visible health status, because Polygonatum rhizomes vary significantly in shape and quality type, and some forms have better comprehensive quality than others (Hu et al., 2022). Agronomic indicators should include unit yield potential and plant vigor, with stem diameter retained as a practical proxy indicator because of its positive correlation with yield (Liao et al., 2023). Chemical indicators should center on polysaccharides as a recognized quality-evaluation index, while also incorporating saponins and flavonoids as candidate quality markers for Polygonatum rhizomes. This combined approach is more suitable than relying on a single index, because metabolite composition changes across years and seasons and cannot be fully represented by polysaccharide content alone (Pu et al., 2024).

In commercial practice, rhizomes can be divided into at least three grades. First-grade rhizomes should come from authenticated, well-adapted, disease-free mother stocks and have uniform morphology, strong fullness, intact buds, and high polysaccharide and saponin levels (Shi et al., 2024). Second-grade rhizomes may show moderate

variation in form or composition but still meet the threshold for medicinal processing or propagation use (Cheng et al., 2023; Liao et al., 2023). Lower-grade rhizomes or younger fractions should be diverted to comprehensive utilization pathways, since young rhizomes still contain substantial polysaccharides and similar biological activity to mature material. To support standardization at scale, rapid non-destructive methods such as NIRS should be incorporated into grading, and the broader quality system should connect variety, environment, metabolite profile, and efficacy so that commercialization standards remain aligned with standardized cultivation and harvesting practice (Pu et al., 2024).

5 Storage and Vigor Maintenance Techniques of Rhizomes

5.1 Key postharvest treatment and sterilization/anti-decay techniques

After harvest, *P. sibiricum* rhizomes should first undergo careful cleaning, wound reduction, and surface disinfection, because rhizome propagules are highly vulnerable to postharvest loss from soil-borne pathogens, pests, and mechanical injury during the storage interval before replanting (Stephen et al., 2023). In stored *P. sibiricum* tubers, a newly reported postharvest rot reached nearly 40% incidence in a storage facility, with lesions expanding from light brown spots to extensive tissue decomposition, confirming that decay control is a central storage problem rather than a secondary one. The causal agent was identified as the *Fusarium solani* species complex, and disease was reproduced after inoculation under 25 °C and 80% relative humidity, indicating that warm and humid storage conditions strongly favor postharvest rot development. For pathogen isolation from diseased *P. sibiricum* tubers, infected tissues were surface-sterilized in 75% alcohol for 90 s followed by 3% hydrogen peroxide for 30 s, providing a directly documented sterilization sequence relevant to rhizome sanitation workflows.

Anti-decay management should also combine biological or physical pretreatments with subsequent low-stress storage. In ginger, seed-rhizome treatment with *Trichoderma harzianum* improved later germination and field performance, especially when paired with a zero-energy cool chamber, showing that pre-storage biocontrol treatment can translate into stronger post-storage vigor. A fungicide treatment also formed part of the tested protection strategies, indicating that both chemical and biological disinfection routes are used for rhizome preservation. Physical pretreatment can also reduce storage injury: ginger rhizomes cleaned, air-dried, and dipped in 45 °C hot water before 5 °C storage showed reduced browning at a 5 min treatment and improved retention of some phytochemical traits, consistent with a preconditioning effect against chilling stress (Shukor et al., 2023). For *P. sibiricum*, this supports a postharvest sequence of sorting out diseased rhizomes, cleaning and drying, minimizing wounds, applying a validated disinfection or biocontrol treatment, and then transferring material promptly into an appropriate storage environment rather than holding it under ambient warm-humid conditions.

5.2 Comparison and applicability of storage methods such as sand storage and cold storage

Different storage methods preserve rhizomes through different mechanisms, so their applicability depends on whether the production goal is short-term sowing viability, delayed sprouting, germplasm conservation, or low-cost farm storage. Traditional matrix-based storage can reduce moisture loss and decay: in ginger, sand, sawdust, and perforated polythene were associated with better sprouting because of lower decay and moisture loss, while pit storage could lose 25%-30% of rhizomes to rot (Stephen et al., 2023). Turmeric studies similarly showed that storage materials significantly reduced postharvest losses relative to no storage material, with straw minimizing weight loss, shrinkage, insect incidence, and rotting, likely by creating an insulated, moisture-retentive microenvironment unfavorable to pests and pathogens (Maharjan and Dhakal, 2025). For field planting material, shade-plus-mulch and tree-shade systems also performed well: turmeric mother rhizomes stored under shade tree plus mulch reached 88.4% sprouting and 94.53% viability, and ginger seed rhizomes stored under tree shade or in mulched pits achieved about 85% viability.

Cold storage is more suitable when the objective is to extend storage duration while maintaining viability, but its success depends on temperature range and rhizome type. In *Miscanthus giganteus*, cold storage had no negative effect on viability, growth, or rhizome carbohydrate and mineral concentrations, and effectively extended planting time. In vitro systems show even stronger conservation potential: encapsulated microrhizomes of *Acorus calamus*

stored in darkness at 10 °C had 100% survival after 1, 3, or 6 months and 80% survival after 12 months, while *Iris pallida* plantlets could be cold-preserved at 4 °C for up to 90 days without significant damage (Meucci et al., 2024). However, refrigeration is not universally superior for fresh seed rhizomes. In ginger, refrigerator storage produced the lowest sprouting and highest weight loss, while pit-sawdust or clay-pot storage performed better over three months (Stephen et al., 2023). For *P. sibiricum*, this implies that sand or mulch-based storage is more practical for short-term nursery use under farm conditions, whereas controlled low-temperature storage is more appropriate for elite propagules, staggered planting, or in vitro conservation, provided temperature and humidity are tightly regulated (De Vitis et al., 2020).

5.3 Environmental regulation and mechanisms for maintaining rhizome vigor

The maintenance of rhizome vigor depends on regulating temperature, moisture, gas exchange, and storage duration so that reserve depletion and pathogen growth are both minimized. Rhizomes remain physiologically active during storage, and temperature strongly affects reserve consumption: in switchgrass, storage at 25 °C for 14 days increased rhizome respiration 5.3-fold relative to 5 °C and depleted starch by 30% (Tejera-Nieves and Walker, 2023). This shows why warm storage rapidly consumes stored carbohydrates that are needed for later regrowth. At the same time, moisture must not be allowed to fall excessively. In small white ginger, storage in an air-conditioned room gave the best 4-month performance, and viability declined when rhizome moisture dropped below 80% (Melati and Rusmin, 2019). Moisture-retentive coverings also help: weekly wetting kept switchgrass rhizomes moist without visible fungal infection during storage, and straw-based turmeric storage reduced physiological weight loss and shrinkage (Maharjan and Dhakal, 2025).

The physiological basis of vigor maintenance is the conservation of non-structural carbohydrate reserves and the suppression of stress injury without triggering uncontrolled growth. Rhizomes function as essential storage organs whose carbohydrates support regrowth after stress, and drought experiments showed that plants conserved rhizomes and maintained carbohydrate concentrations rather than sacrificing them (Lubbe et al., 2023). In cold in vitro conservation, slow-growth storage works by preserving tissue maintenance while suppressing energy-intensive organogenesis, and additives such as sucrose, antioxidants, sorbitol, mannitol, spermidine, and calcium pantothenate improved viability and stress tolerance during storage (Meucci et al., 2024; Khan et al., 2025). Storage duration remains a hard limit, because prolonged storage generally lowers phytochemical and antioxidant quality in rhizomes even when low temperature extends shelf life (Nawaz et al., 2025). Therefore, maintaining *P. sibiricum* rhizome vigor requires a storage environment that is cool but not injurious, humid but not saturated, ventilated yet protected, and short enough to limit reserve decline and pathogen buildup while preserving the bud's capacity for rapid post-storage sprouting (Maharjan and Dhakal, 2025).

6 Key Technologies for Standardized Seedling Production

6.1 Site selection and environmental optimization of seedling production bases

Seedling production bases for *Polygonatum sibiricum* should be located in areas where soil reaction, microbial environment, and local climate jointly support rhizome growth and quality. In *Polygonatum*, genotype adaptability is closely related to rhizosphere conditions, especially soil pH, and the highest rhizome fresh weight and polysaccharide content were associated with soils at pH 7.48-7.95, whereas poor yield and quality were linked with pathogenic microorganisms such as *Pseudomonas*, *Fusarium*, *Neocosmospora*, and *Tausonia* (Shi et al., 2024). This indicates that site selection should avoid acidic, poorly drained, pathogen-prone soils and instead prioritize plots with neutral to slightly alkaline reaction, good aeration, and a stable beneficial microbial community. More broadly, seedling quality depends on matching nursery stock to future site conditions, because defining and producing seedlings according to site-specific requirements improves establishment success (Guimarães et al., 2024).

Environmental optimization within the base should therefore integrate topography, drainage, and local microclimate rather than focusing on a single factor. Nursery stock quality is shaped by both morphology and physiology, and inappropriate cultural or environmental management can produce inferior seedlings that later perform poorly in the field. Because root growth and stress resistance are highly sensitive to temperature and

water status, base layout should favor areas with controllable irrigation, rapid drainage after rainfall, and moderated temperature fluctuations. The practical target is a nursery environment that reduces abiotic stress during early establishment while allowing seedlings to develop balanced shoot-root architecture and adequate acclimation capacity before transplanting.

6.2 Substrate formulation and precise water-fertilizer management techniques

Substrate formulation for *P. sibiricum* seedlings should emphasize porosity, moisture buffering, and support for root-system development, because root morphology is a major determinant of seedling quality and later field performance. Across nursery studies, root volume, total root length, root surface area, root dry mass, and the number of first-order lateral roots repeatedly emerged as effective indicators of seedling quality (Robonen et al., 2023). Root restriction or poor substrate structure reduces the seedling's capacity for water and nutrient uptake, while better-developed root systems support higher biomass accumulation under stress (Wu et al., 2022). For this reason, substrate mixtures should be loose, well-aerated, and structurally stable, so that rhizome-derived seedlings can produce fibrous roots and maintain an appropriate shoot-root balance.

Water and fertilizer management should be precise because both deficiency and excess quickly impair photosynthesis, growth, and physiological quality. Nitrogen deficiency in rice seedlings reduced CO₂ assimilation, stomatal conductance, and chlorophyll content, while impairing the photosynthetic electron transport chain and activating oxidative-stress defenses. Excessively severe water stress likewise reduced growth and photosynthetic traits in multiple seedling systems, while moderate control of water status is central to vigor maintenance and stress conditioning (Shin et al., 2021; Wu et al., 2022). In practical nursery management, this supports small-dose, stage-specific fertilization and moisture regulation based on substrate water-holding status, with routine observation of chlorophyll status, biomass allocation, and root growth as feedback indicators (Zhang et al., 2024; Baha et al., 2025). System optimization should favor steady nutrient supply and avoidance of prolonged saturation, because these conditions better preserve root activity and reduce the risk of weak, top-heavy seedlings (Robonen et al., 2023; Lee et al., 2026).

6.3 Shading regulation and integrated green pest and disease control system

Shading regulation should be used to stabilize the microenvironment, but not to the extent that it suppresses biomass formation and physiological performance. Light quality and intensity strongly regulate seedling photosynthetic efficiency, pigment accumulation, oxidative status, and biomass yield. In alfalfa seedlings, combined red and blue light improved Fv/Fm, SPAD, chlorophyll content, and biomass, while single red light increased oxidative stress indicators (Rahman et al., 2025). At the same time, excessive shade can reduce dry-matter accumulation by inhibiting photosynthesis and disturbing agronomic traits (Zhang et al., 2024). For *P. sibiricum* nurseries, this means shading should be dynamically adjusted to reduce heat and water stress in sensitive periods while maintaining sufficient photosynthetic input for robust rhizome and root formation.

Green pest and disease control should combine environmental prevention, substrate hygiene, and beneficial-microbe management. In *Polygonatum*, poor yield and quality are associated with pathogenic rhizosphere microorganisms including *Fusarium*, while better performance is associated with beneficial groups such as *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Talaromyces* (Robonen et al., 2023). More generally, beneficial microbial inoculation in nursery substrates improved germination, chlorophyll content, shoot length, root length, and seedling vigor in ornamental crops (Liu et al., 2023). Compost-based and bioinoculant-based systems can also contribute pathogen suppression, nutrient solubilization, and biostimulation, making them suitable components of an integrated green management system (Yu et al., 2025). A standardized *P. sibiricum* seedling base should therefore use healthy propagation material, sanitized substrates, rational shading and irrigation, and biologically oriented disease prevention to reduce dependence on high-intensity chemical control while maintaining uniform, vigorous seedlings for release.

7 Seedling Quality Evaluation and System Optimization

7.1 Evaluation system for morphological and root-related indicators

The morphological evaluation system for *P. sibiricum* seedlings should use easily measured nursery traits as the first screening layer, with seedling height, root-collar diameter, shoot biomass, root biomass, total biomass, and

root-shoot balance as core indicators. Among these traits, root-collar diameter is the most consistently useful single index, because it correlates strongly with destructive quality traits and with composite indices such as Dickson quality index across multiple species (Guimarães et al., 2024). Height should not be used alone, because its interpretive value improves only when combined with diameter and biomass-allocation traits (Robonen et al., 2023). Accordingly, a practical nursery standard for *P. sibiricum* should record seedling height, basal diameter, shoot fresh and dry weight, root fresh and dry weight, total dry matter, and shoot-root ratio, and then calculate integrated indices such as H/D, S/R, and DQI to reduce misclassification caused by any single trait (Lee et al., 2026).

Root-related traits should form the second core module of the evaluation system, because aboveground morphology alone is often an incomplete predictor of later establishment. The most informative root indicators are total root length, root surface area, root volume, root dry mass, fibrosity, and the number of first-order lateral roots, all of which reflect absorptive capacity and establishment potential (Robonen et al., 2023). Diameter remains useful here as well, since initial stem diameter predicted later root volume, root area, and root dry mass better than shoot length or lateral-root number in red oak seedlings. For operational detection, root systems can be scanned and quantified by image-analysis platforms such as WinRHIZO, which have been used to measure root length, surface area, volume, average diameter, root tips, forks, and crossings under seedling stress and quality studies (Wu et al., 2022; Mahmood et al., 2022). Because nursery morphology does not always translate directly into field growth, especially across different sites or species, the morphological system for *P. sibiricum* should be calibrated as a target seedling standard linked to local planting conditions rather than treated as a universal threshold (De Melo et al., 2018; Guimarães et al., 2024).

7.2 Physiological and biochemical indicators and vigor detection methods

Morphological indices should be complemented by physiological and biochemical indicators, because seedling quality also depends on physiological readiness, water relations, nutrient status, carbohydrate reserves, and photosynthetic function. Among rapid vigor indicators, chlorophyll-related indices, SPAD value, photosynthetic rate, stomatal conductance, and chlorophyll fluorescence are especially useful because they respond sensitively to nutrient deficiency and environmental stress (Liu et al., 2023). The maximum quantum efficiency of PSII, F_v/F_m , is a particularly valuable indicator: it declined under chilling stress in corn, under nitrogen deficiency in rice, and was incorporated into recent integrated morphophysiological seedling quality indices (Wu et al., 2022; Lee et al., 2026). SPAD and chlorophyll content also track vigor well, since they were identified among key indicators in drought-resistance evaluation and multimodal seedling grading models (Liu et al., 2023; Yu et al., 2025).

Biochemical vigor detection should focus on reserve and stress-metabolism indicators, especially carbohydrates, proline, malondialdehyde, and antioxidant-enzyme activities such as SOD, CAT, POD, and APX. Under drought or chilling, vigorous seedlings typically maintain better growth and photosynthesis while activating osmotic adjustment and antioxidant defense, whereas sensitive seedlings show stronger membrane peroxidation and growth suppression (Wu et al., 2022). Proline accumulation, SOD activity, and controlled MDA response were useful discriminators between drought-resistant and drought-sensitive cotton lines, and MDA plus soluble sugars were principal predictors in *Gleditsia* drought-response analysis (Baha et al., 2025). For direct vigor testing before transplanting, root growth potential, electrolyte leakage, budbreak response, carbohydrate concentration, and cold-hardiness tests remain informative because they integrate multiple seedling subsystems rather than measuring one material trait in isolation. In system optimization, these physiological and biochemical measures should be used to verify or correct morphology-based grading, not to replace it, because performance tests are often more predictive but also more laborious.

7.3 Stress resistance evaluation and grading standards for seedling release from nursery

Seedlings released from the nursery should meet explicit stress-resistance standards, because seedling quality is ultimately defined by the ability to survive environmental stress and sustain subsequent growth. Stress-resistance evaluation for *P. sibiricum* should therefore include low-temperature, drought, and transplanting-related tolerance, assessed through a combination of growth retention, leaf morphology, photosynthetic traits, water status,

membrane-damage markers, and antioxidant defense indices (Mahmood et al., 2022). A single indicator is not sufficient for this task, because drought and other stress responses are controlled by multiple traits and pathways; comprehensive evaluation using PCA, correlation analysis, grey correlation, drought indices, or entropy-weight methods gives a more objective basis for classification (Baha et al., 2025). Recent grading studies in maize further show that multimodal phenotypic systems can classify seedling quality with high accuracy, and that a reduced set of key indices such as plant height, stem diameter, leaf area, root volume, and shoot and root biomass can support efficient automated grading (Zhang et al., 2024; Yu et al., 2025).

For nursery release of *P. sibiricum*, a three-level standard is practical. First-grade seedlings should have balanced morphology, well-developed fibrous roots, high DQI or comparable composite quality scores, stable chlorophyll fluorescence and SPAD values, and low membrane-damage indicators under routine hardening tests (Lee et al., 2026). Second-grade seedlings may show moderate variation in size or biomass allocation but should still maintain acceptable root morphology, photosynthetic activity, and biochemical stability for field establishment (Baha et al., 2025). Rejected seedlings should include weak, top-heavy, root-deficient, physiologically unstable, or stress-sensitive individuals showing poor root volume, unbalanced shoot-root ratio, depressed fluorescence traits, or excessive MDA accumulation (Shin et al., 2021). In practice, release standards should be validated against local field performance and revised iteratively, because seedling quality indices are species- and site-dependent rather than universally transferable (Robonen et al., 2023).

8 Conclusion

This paper systematically examines the construction of a technical system for rhizome propagation and high-quality seedling production of *Polygonatum sibiricum*, covering the biological basis of rhizome propagation, propagation techniques, rhizome formation and harvest standards, storage and vigor maintenance, standardized seedling production, and seedling quality evaluation. The findings indicate that the rhizome of *P. sibiricum* is not only the main medicinal part but also the core organ for vegetative propagation. Its segmented structure, bud development, nutrient reserves, and environmental responses jointly determine propagation efficiency and seedling quality. Compared with seed propagation, rhizome propagation has the advantages of a shorter production cycle, stable trait inheritance, and suitability for rapid multiplication of elite germplasm, making it an important technical pathway for large-scale cultivation and standardized seedling production of *P. sibiricum*.

In terms of technical system construction, rhizome propagation of *P. sibiricum* should be based on the selection of superior mother plants and the quality control of healthy rhizome sources. Whole-rhizome propagation, division propagation, segment propagation, bud-section propagation, and tissue culture rapid propagation should be integrated to establish a hierarchical propagation model for different production purposes. Meanwhile, rhizome harvesting should comprehensively consider growth duration, seasonal differences, rhizome morphology, and the accumulation of active constituents, so as to establish grading standards based on morphological indicators, agronomic traits, and chemical components. During postharvest storage, cleaning, disinfection, anti-decay treatment, suitable temperature and humidity control, and pathogen management should be adopted to maintain rhizome vigor, reduce decay and nutrient depletion, and provide stable propagation materials for subsequent seedling production.

High-quality seedling production of *P. sibiricum* also depends on a standardized nursery management system, including appropriate site selection, substrate optimization, precise water-fertilizer regulation, shading management, and green pest and disease control. Before nursery release, a comprehensive quality evaluation system should be established based on morphological indicators, root-related indicators, physiological and biochemical traits, and stress resistance assessment, while grading standards should be dynamically revised according to different cultivation regions and production goals. Overall, constructing a technical system for rhizome propagation and high-quality seedling production of *P. sibiricum* can improve seedling uniformity and transplanting survival rate, promote the conservation and rapid multiplication of elite germplasm, reduce dependence on wild resources, and provide technical support for the standardized, large-scale, sustainable, and high-quality development of the *P. sibiricum* industry.

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Feature Review

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Conservation of Rare Medicinal Plant Resources and Artificial Replacement Cultivation Pathways of *Anoectochilus roxburghii*

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Abstract *Anoectochilus roxburghii* is an important rare medicinal orchid in China, with medicinal, health-promoting, edible, and ornamental value. It is rich in various active components, including kinsenoside, flavonoids, polysaccharides, phenolic acids, and nucleosides, and shows broad pharmacological activities such as hepatoprotective, hypoglycemic, anti-inflammatory, antioxidant, and immunomodulatory effects. However, long-term overharvesting, habitat destruction, slow growth, and weak natural propagation have led to the continuous decline of wild resources, seriously restricting sustainable industrial development. Artificial replacement cultivation has become an important approach to relieving resource pressure, ensuring raw material supply, and promoting industrial development. This study discusses the significance of wild resource conservation of *A. roxburghii* and analyzes key technical pathways for artificial replacement cultivation, including tissue culture and rapid propagation, optimization of artificial cultivation models, precise environmental regulation, active-component formation patterns, and construction of a whole-process quality control system. Meanwhile, it summarizes development pathways for standardized production system construction, coordinated resource conservation and industrial development, industrial chain extension, and high-value utilization. It also analyzes current problems, including insufficient utilization efficiency of superior germplasm, unstable quality of artificially cultivated materials, and an incomplete standardized industrial system, and proposes optimization strategies such as strengthening superior germplasm innovation, improving whole-process quality control, promoting standardized production, and advancing scientific and technological innovation. In the future, wild resource conservation and artificial replacement cultivation should be promoted collaboratively, with scientific and technological innovation driving high-quality seedling propagation, quality improvement, and industrial upgrading, thereby achieving coordinated development of resource conservation, artificial cultivation, and high-quality industrial development of *A. roxburghii*, and providing a theoretical basis and practical reference for the sustainable utilization of rare medicinal plant resources.

Keywords *Anoectochilus roxburghii*; Artificial replacement cultivation; Germplasm resource conservation; Quality control; Standardized production

1 Introduction

Anoectochilus roxburghii, also known as Jinxianlian, is widely recognized in Asia as an important medicinal and edible plant and is also valued as an ornamental species. Phytochemical studies indicate that it contains polysaccharides, flavonoids, glycosides, alkaloids, organic acids, steroids, triterpenes, volatile compounds, and the characteristic active constituent kinsenoside, which together underpin its broad medicinal value (Hou et al., 2025). Pharmacological and clinical-use reports show that this species has been used for diabetes, hyperlipidemia, liver disease, inflammatory disorders, cancers, chronic hepatitis B, hyperuricemia, and related conditions, with reported activities including antidiabetic, hepatoprotective, antioxidant, anti-inflammatory, immunomodulatory, renal-protective, and antineoplastic effects (Wang et al., 2022). In addition to medicinal use, *A. roxburghii* has entered food, nutraceutical, cosmetic, and functional health-product markets, and has been developed into soups, beverages, jelly, face masks, soap, and other products, reflecting a continuing expansion from traditional herbal use toward diversified industrial applications (Wang et al., 2018). Patent mining further shows that current technological development is concentrated in pharmaceutical preparations and food products, especially downstream applications such as anti-inflammatory, anti-infective, analgesic, and antipyretic products, while companies remain the main research and development actors in this field (Shi et al., 2023). At the same time,

industrial development still faces important bottlenecks, including slow variety breeding, insufficient quality-control systems, weak product innovation, limited brand recognition, and the absence of unified evaluation standards across regions; notably, *A. roxburghii* has not yet been included in the 2020 Chinese Pharmacopoeia, which constrains standardization, safety evaluation, and high-quality industrial development (Hong et al., 2016; Zou et al., 2025).

Despite this high value, wild *A. roxburghii* resources have undergone persistent depletion for more than two decades. Early resource surveys already emphasized the need for rational preservation and exploitation, while later field investigations in Xishuangbanna found that intensive collection had left the species only sporadically distributed in relatively inaccessible places, making artificial reproduction a practical necessity. More recent studies consistently attribute population decline to overexploitation, habitat destruction, ecological deterioration, slow growth, low seed germination, low natural propagation rates, and strict habitat requirements (Wang et al., 2018; Hou et al., 2025). Surveys in central Vietnam similarly recorded only 123 individuals across 9 of 20 survey lines in Bach Ma National Park, illustrating the scarcity and uneven distribution of extant populations even in protected landscapes (Ho et al., 2025). This conservation pressure is now formally recognized: *A. roxburghii* has been described as vulnerable, near-threatened, or endangered in international and national conservation frameworks, and has been listed as a National Level II Protected Plant in China (Wang et al., 2022; Hou et al., 2025). Its vulnerability is intensified by ecological specialization. The species depends strongly on precise light conditions for growth, photosynthesis, and flavonoid accumulation, and its sensitivity to abiotic stress suggests limited buffering capacity under environmental change. Climate modeling therefore indicates that, alongside direct human disturbance, future shifts in temperature and precipitation may further alter suitable habitats, making in situ conservation, germplasm rescue from contracting populations, and regionally guided artificial cultivation increasingly urgent.

This study will explore the key technical pathways for the coordinated development of *Anoectochilus roxburghii* resource conservation and artificial replacement cultivation. Against the background of continuous wild resource decline and increasing market demand, artificial replacement cultivation has become a core approach to relieving resource pressure, ensuring the supply of high-quality raw materials, and promoting sustainable industrial development. In recent years, tissue culture and in vitro rapid propagation technologies have enabled efficient multiplication, genetic consistency, and disease-free seedling production, while large-scale seedling systems and protocorm-like body (PLB) propagation have significantly improved propagation efficiency and transplant survival, providing important technical support for germplasm conservation and industrial production. Meanwhile, technologies such as light-environment regulation, wild-imitated cultivation, endophyte regulation, and under-forest ecological cultivation can further promote active-component accumulation, improve medicinal quality, and strengthen the connection between cultivated materials and wild-type quality. Based on this, this paper systematically analyzes the significance of wild resource conservation of *A. roxburghii*, discusses key technologies including seedling propagation, cultivation model optimization, precise environmental regulation, medicinal quality formation, and whole-process quality control, summarizes pathways for standardized production, coordinated industrial development, and high-value utilization, and proposes optimization strategies for current problems in artificial replacement cultivation, aiming to provide theoretical and practical references for resource conservation, cultivation technology optimization, and high-quality industrial development of *A. roxburghii*.

2 Significance of Wild Resource Conservation

2.1 Genetic value

Wild germplasm resources of *Anoectochilus roxburghii* have irreplaceable genetic value because they preserve the full range of natural variation formed under long-term habitat selection (Priyanka et al., 2021; Yadav et al., 2024). As an endangered medicinal orchid with slow growth, low natural propagation, and narrow habitat requirements, the loss of wild populations would directly reduce the species' adaptive and evolutionary potential. Climate contraction is especially important for peripheral populations, because populations at distribution margins can contain unique adaptive variation and are at greatest risk of disappearance, making targeted germplasm rescue

necessary (Hou et al., 2025). More broadly, work on wild plant germplasm shows that natural populations are valuable gene pools for future breeding, stress resistance, and long-term improvement, a principle that is highly relevant to *A. roxburghii* conservation.

Recent molecular studies also show why wild germplasm should be treated as a strategic breeding resource rather than only a protected object. Transcriptome assembly in *A. roxburghii* generated 138 385 unigenes and identified 44 045 SSRs, providing marker resources for assessing diversity across accessions and supporting more effective breeding and conservation (Zhang et al., 2024). DNA barcode work further indicates that matK and ITS can support reliable species identification, with ITS offering better phylogenetic resolution, which is useful for germplasm authentication and genetic resource management (Nhàn, 2025). At the trait level, tetraploid materials produce more major secondary metabolites than diploids, showing that conserving genetically distinctive materials can directly expand medicinal and breeding value (Zhang et al., 2025).

2.2 Ecosystems and population restoration

Protecting the native ecosystem is fundamental because *A. roxburghii* depends on highly specific forest microhabitats rather than broad environmental tolerance. Field and distribution studies show that the species occurs mainly under evergreen or semi-deciduous broadleaf forest with high humidity, large canopy cover, and limited disturbance, while over-collection, deforestation, and forest conversion have sharply reduced wild occurrence (Ho et al., 2025). Its growth, photosynthesis, and flavonoid accumulation are strongly affected by light conditions, and its narrow ecological amplitude indicates limited buffering capacity under environmental change. Because stable future refugia are predicted mainly in Fujian, Guangdong, Guangxi, Yunnan, and Guizhou, these areas should remain priorities for in situ protection and reserve-gap assessment (Hou et al., 2025).

Population restoration should therefore combine habitat protection with scientifically guided reinforcement. Surveys in Bach Ma National Park found only 123 individuals across 9 of 20 survey lines, usually in small clusters of 4-15 plants, illustrating both rarity and fragmentation (Ho et al., 2025). Earlier regional studies likewise proposed ecological study, tissue-culture multiplication, and wild tending to increase population size and provide a basis for recovery. Restoration also needs to account for stress biology and disease control: drought severely threatens the species, candidate drought-resistance genes have now been identified for molecular breeding, and soft rot can reduce cultivated yield by 70%~80%, which means healthy reintroduction materials require both abiotic and biotic resilience (Xing et al., 2022; Jiang et al., 2025).

2.3 Conservation systems and sustainable utilization

A sustainable conservation system for *A. roxburghii* should integrate in situ protection, ex situ germplasm preservation, dynamic monitoring, improved-variety propagation, and regulated industrial utilization. Industry analyses identify weak wild-resource protection, slow variety breeding, and insufficient quality-system research as key constraints, and recommend stronger breeding-resource protection, dynamic monitoring, and improved propagation systems (Hong et al., 2016). General germplasm research likewise shows that effective conservation requires collection, storage, analysis, documentation, and exchange, using complementary methods such as slow-growth culture, cryopreservation, DNA banks, botanical gardens, and genetic reserves (Priyanka et al., 2021). For rare plants, in situ conservation is generally preferred, but ex situ systems are essential to prevent genetic loss and to maintain material for future recovery and breeding (Yadav et al., 2024).

Sustainable utilization depends on replacing destructive wild harvesting with high-quality artificial cultivation that still maintains medicinal characteristics. Large-scale micropropagation protocols now achieve shoot induction of 91.67%, rooting of 93.33%, and transplant survival of 90.2%, while tetraploid propagation systems also support high rooting and acclimatization rates, showing that ex situ multiplication can relieve harvest pressure on wild populations (Zhang et al., 2025). Tissue culture is already regarded as the main seedling-raising method because it enables rapid, genetically consistent, disease-free propagation and supports germplasm protection (Li and Li, 2025). At the same time, under-forest and wild-imitated cultivation are important because they better align production with ecological requirements, and wild-imitated systems can shift endophytic communities toward

wild-like states while increasing kinsenoside-associated microbial functions, offering a stronger path toward coordinated conservation and utilization.

3 Artificial Cultivation Technical Pathways

3.1 Seedling propagation technology of *Anoectochilus roxburghii*

Seedling propagation is the primary technical foundation for artificial replacement cultivation of *Anoectochilus roxburghii*, because conventional propagation is constrained by low natural regeneration and unstable seedling supply (Wang et al., 2022). Tissue culture has therefore become the main seedling-raising route, especially for rapid multiplication, germplasm conservation, and standardized industrial production (Li and Li, 2025). Large-scale nodal-segment culture systems have already achieved 91.67% shoot induction on 1/2 MS with 1.5 mg/L BA, a proliferation coefficient of 4.33 on 1/2 MS with BA, Kn, and NAA, and 93.33% rooting on medium containing NAA, IBA, and banana mash. After acclimatization, these regenerated plantlets reached 90.2% survival in a sand:peat substrate covered with live moss, showing that tissue-cultured seedlings can enter ex vitro production at high efficiency.

Recent studies further show that protocorm and protocorm-like body systems can markedly increase propagation efficiency and broaden the usable germplasm base. An optimized PLB induction, proliferation, regeneration, and rooting protocol produced an 89% PLB induction rate, a 400% secondary PLB proliferation rate, 10.5 shoots per PLB mass, and 98% rooting, while all plantlets survived acclimation (Wang et al., 2022). A newer floral-bud/protocorm route achieved 83.0% protocorm regeneration, 50.33 PLBs per explant, 35 shoots per PLB cluster, and 85% greenhouse survival after 12 months, while kinsenoside content continued to increase during greenhouse cultivation (Luan et al., 2026). For tetraploid materials, B5 medium plus 1 mg/L BA and 0.05 mg/L NAA improved shoot proliferation, red light raised shoot induction to 92%, and MS with 1.0 mg/L IBA plus 1.0 mg/L NAA produced 92% rooting, followed by 80% survival after pot transfer (Zhang et al., 2025).

3.2 Optimization of artificial cultivation models for *Anoectochilus roxburghii*

Beyond seedling production, the success of artificial replacement cultivation depends on selecting cultivation models that balance survival, biomass accumulation, medicinal quality, and ecological similarity to wild habitats. Early practice showed that under-forest cultivation was proposed specifically to address resource exhaustion by using shaded forest environments to simulate native ecological conditions. Substrate-based field domestication studies on Dinghushan Mountain found that peat: perlite at 4:1 or peat:sand:sawdust at 3:1:1 supported 94.3%-97.6% seedling survival and good growth under natural conditions. Quality-oriented cultivation analysis likewise suggested that high-quality production was associated with MS medium and a humus soil:river sand:perlite substrate of 600:200:200, although that conclusion still requires broader validation (Xu et al., 2017). These results show that matrix design is not a minor management variable, but a core determinant of establishment success and product quality.

Current model optimization is moving from simple soil cultivation toward wild-imitated, microbe-assisted, and hydroponic or soilless systems. Wild-imitated cultivation changed the endophytic community within three months so that it converged toward wild-tending plants, and bacterial diversity was positively associated with kinsenoside content, especially through *Burkholderia-Caballeronia-Paraburkholderia*. Arbuscular mycorrhizal inoculation with *Glomus intraradices* improved root architecture, biomass, polysaccharides, flavonoids, and stalk-rot resistance, supporting a model in which beneficial fungi are integrated into production systems rather than treated as incidental (Gu et al., 2025). Hydroponic culture systems also show promise: in vitro Erlenmeyer-flask culture with cotton-layer substrate and SH medium improved shoot growth, while ex vitro hydroponics with cotton-layer substrate and 1/2 SH solution gave the best biomass and kinsenoside accumulation (Luan et al., 2025). More broadly, soilless cultivation offers a pathway for precise rhizosphere regulation, higher nutrient-use efficiency, and microbiome engineering, though cost and substrate sustainability remain practical constraints (Tuxun et al., 2025).

3.3 Precise environmental regulation technology for *Anoectochilus roxburghii* cultivation

Because *A. roxburghii* has narrow ecological amplitude, precise environmental regulation is essential for replacing wild collection with stable artificial production. Light regulation is one of the clearest examples. Orthogonal light experiments showed that light intensity, red:blue ratio, and photoperiod all significantly affected growth and metabolite accumulation, with $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, red:blue=2:1, and a 10 h photoperiod favoring growth and polysaccharides, while $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, red:blue=1:1, and a 14 h photoperiod favored flavonoids (Chen et al., 2021). Supplemental blue light also promoted leaf number, stem diameter, biomass, chlorophyll a, flavonoids, and polyphenols, whereas yellow light increased soluble sugar and polysaccharides (Wang et al., 2018). During in vitro propagation, light effects are stage-specific: darkness or weak light favors PLB proliferation, but light is required for PLB differentiation, and red light can enhance tetraploid shoot proliferation (Figure 1) (Wang et al., 2022; Zhang et al., 2025).

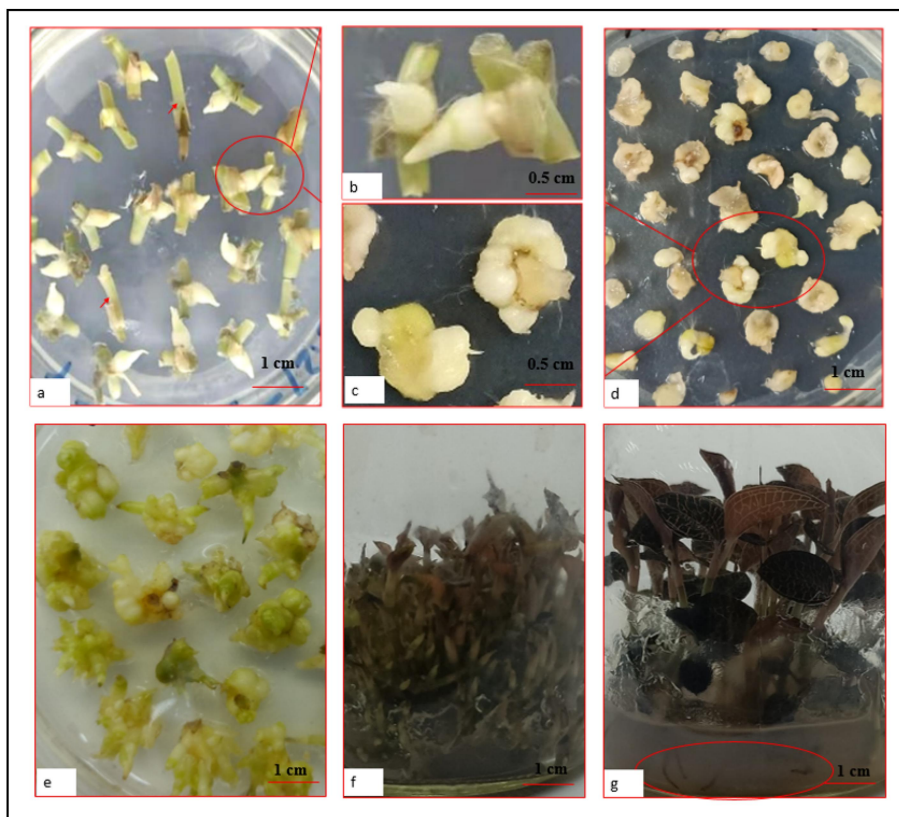


Figure 1 Induction, proliferation, and regeneration of *A. roxburghii* PLB (Adopted from Wang et al., 2022)

Image caption: (a) Induction of *A. roxburghii* PLBs. The arrows indicate stem nodes near apical shoot;. (b) Magnification ($4\times$) of the selected area in panel a; (c) Magnification ($4\times$) of the selected area in panel d; (d) Secondary PLB induction; (e) Mastoid PLB mass; (f) Shoot formation; (g) Root formation (the roots are within the circled region) (Adopted from Wang et al., 2022)

Temperature, humidity, shading, and planting density also require fine control across different cultivation stages. Under Dinghushan conditions, optimal growth was observed at $23\sim 28^\circ\text{C}$, pH 4.75, and about 90% relative humidity, with shade-net use and avoidance of excessive substrate moisture. Shading studies in micropropagated *Anoectochilus* plantlets found that mild to medium shade generally supported biomass production and faster growth, while species responses still differed (Chen et al., 2017). Newer work on shade-tolerant responses in *A. roxburghii* showed that moderate dense planting improved stem diameter, leaf area, SOD activity, and stem lignin, while increasing far-red light reduced stalk-rot incidence and strengthened defense-related enzyme activity rather than triggering a typical shade-avoidance response (Guo et al., 2025). Precise regulation also extends to the transplant stage, where hormone balance, osmotic adjustment, antioxidant activation, substrate improvement, and later-stage light management jointly determine root-system recovery and seedling establishment after tissue culture (Li and Li, 2025).

4 Quality Formation and Quality Control

4.1 Accumulation patterns and influencing factors of active components in *Anoectochilus roxburghii*

The medicinal quality of artificially cultivated *Anoectochilus roxburghii* is determined by the accumulation of multiple active components, chiefly kinsenoside, flavonoids, polysaccharides, phenolic acids, and nucleosides, rather than by any single compound (Zou et al., 2025). Kinsenoside is especially important because *A. roxburghii* is its exclusive medicinal source, but its content varies substantially among varieties, production areas, and cultivation periods (Zou et al., 2025). Variety effects are clear: among nine varieties, Hongxiadaye had the highest kinsenoside content, while Xiaoyuanye uniquely contained both kinsenoside and its isomer (Figure 2) (Wang et al., 2025). Regional heterogeneity is equally strong, with targeted and untargeted metabolomics showing large differences in flavonoid profiles among Fujian habitats, and Youxi samples having higher average levels of eight quantified flavonoids despite Yongchun showing the richest unique-metabolite diversity (Lyu et al., 2024).

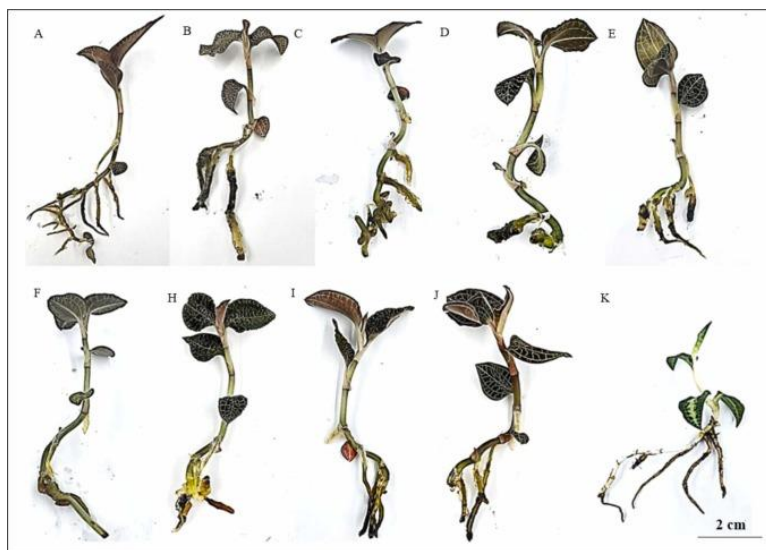


Figure 2 Whole plants of 10 genuine and counterfeit *A. roxburghii* (Adopted from Wang et al., 2025)

Image caption: (A) Caixia, (B) Jianye, (C) J6 Gong, (D) J6 Mu, (E) Dayuanye, (F) Xiaoyuanye, (G) Jinmai, (H) Hongxia, (I) Hongxiadaye, and (J) *G. schlechtendaliana* (Adopted from Wang et al., 2025)

Active-component accumulation is also shaped by developmental stage and culture system. In greenhouse-acclimatized plants from floral-bud-derived propagules, whole-plant kinsenoside rose with cultivation time and peaked at 2.591% after 12 months (Zou et al., 2025). In hydroponic systems, ex vitro cotton-layer and cocopeat systems supplied with 1/2 SH nutrient solution produced higher whole-plant kinsenoside accumulation of 1.24%~1.32% dry weight (Luan et al., 2025). Rhizome bioreactor culture showed that biomass, kinsenoside, and polysaccharide accumulation peaked at 30 days, reaching 2980.5 mg/L and 5 672.9 mg/L for kinsenoside and polysaccharides, respectively, under optimized inoculum, aeration, and light conditions (Wei et al., 2020). At the mechanistic level, transcriptomic analysis linked higher kinsenoside accumulation to elevated expression of *ACAA*, *Hbd*, *CoAT*, *PaaH*, and *UGT* genes, while light-regulation studies further showed that red-blue light activates phenylpropanoid, flavonoid, and kinsenoside biosynthetic pathways (Wang et al., 2025; Luo et al., 2025).

4.2 Regulatory effects of cultivation environment on the medicinal quality of *Anoectochilus roxburghii*

Among environmental factors, light quality is the most consistently validated regulator of medicinal quality in cultivated *A. roxburghii*. Blue film increased fresh weight, leaf area, chlorophyll, antioxidant enzyme activities, polysaccharides, and flavones, whereas red film promoted plant height and phenolic accumulation. Supplemental blue light likewise increased biomass, total flavonoids, and total polyphenols, while yellow light preferentially increased soluble sugars and polysaccharides (Wang et al., 2018). More precise orthogonal optimization showed that light intensity, red:blue ratio, and photoperiod all significantly affect growth and metabolite accumulation, with 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 2:1 red:blue, and a 10 h photoperiod favoring growth and polysaccharides, while 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 1:1 red:blue, and a 14 h photoperiod favored flavonoids (Chen et al., 2021). Recent metabolomic

work further showed that an R2B1 light regime substantially increased amino acids, polyphenols, and kinsenoside, with kinsenoside rising by 81.25% in leaves and 72.24% in stems relative to white light (Wu et al., 2023; Luo et al., 2025).

The cultivation substrate, habitat-like microclimate, and microbial symbiosis also strongly regulate medicinal quality. Habitat metabolomics showed that flavonoid accumulation is significantly associated with temperature and humidity, while morphological performance was better in more humid habitats (Lyu et al., 2024). Practical cultivation studies identified suitable conditions of 23~28 °C, about 90% relative humidity, pH 4.75, and shaded management, with peat-based mixed substrates supporting 94.3%~97.6% seedling survival (Xu et al., 2017). Wild-imitated cultivation increased kinsenoside-associated quality by shifting the endophytic community toward that of wild-tending plants, and bacterial diversity showed a significant positive correlation with kinsenoside content, especially for *Burkholderia-Caballeronia-Paraburkholderia*. Beneficial fungi and bacteria exert similar effects: endophytic fungal strains increased biomass and induced flavonoids, kinsenoside, and polysaccharides, while dual *Bacillus velezensis* inoculation increased fresh weight by 82.6% and 106.6%, raised kinsenoside by 9.33% and 21.65% per gram, and enhanced flavonoids and rhizosphere enzyme activities (Ye et al., 2020; Wei et al., 2020).

4.3 Construction of a whole-process quality control system for *Anoectochilus roxburghii*

A whole-process quality control system for *A. roxburghii* must begin at the source with authenticated germplasm and continue through cultivation, harvesting, processing, storage, and final product evaluation. Current reviews agree that quality is jointly shaped by germplasm, producing area, harvesting, processing, preparation, and storage. Germplasm authentication should combine morphological identification with molecular tools, because SSR markers and DNA barcoding have been proposed for classification, resource preservation, and varietal discrimination (Zou et al., 2025). Because content differs markedly among strains and origins, source control should include elite-variety selection, origin traceability, and chemotype classification rather than simple species confirmation (Wang et al., 2025). During cultivation, quality control should integrate standardized light, substrate, humidity, and microbial-management protocols, since each of these factors measurably alters active-compound accumulation (Xu et al., 2017; Wei et al., 2020; Chen et al., 2021).

Post-harvest control is equally necessary because active components decline during processing and storage. Different drying temperatures affect kinsenoside, total flavonoids, eight individual flavonoids, and six nucleosides (Zou et al., 2025). Among drying methods, vacuum drying at (60±2) °C best preserved appearance, aroma, and active ingredients overall, although freeze-drying better maintained natural appearance and hot-air or microwave drying preserved specific sensory or polysaccharide traits. Storage studies showed that polysaccharides, kinsenoside, and flavonoids all declined over eight months, vacuum packaging better preserved polysaccharides and flavonoids, and kinsenoside degradation was driven mainly by endogenous enzymes rather than packaging method (Wei et al., 2022). At the analytical end, single-index control is inadequate, so multi-component methods such as LC-MS/MS, metabolomics, and integrated phenotype-barcode-chemotype-transcriptome frameworks should be used to establish Q-markers and comprehensive specifications; this is particularly important because *A. roxburghii* still lacks a unified pharmacopoeial standard and consistent cross-provincial evaluation system (Zhang et al., 2022; Wang et al., 2025).

5 Standardized Production and Industrial Pathways

5.1 Construction of a standardized production system for *Anoectochilus roxburghii*

A standardized production system for *Anoectochilus roxburghii* should begin with stable seedling supply, because wild resources are endangered, conventional propagation is inefficient, and industrial cultivation depends on reproducible micropropagation rather than continued wild collection (Hong et al., 2016; Wang et al., 2022). Current evidence already provides workable technical benchmarks for standardized seedling production: nodal-segment culture achieved 91.67% shoot induction, a proliferation rate of 4.33, 93.33% rooting, and 90.2% transplant survival under optimized media and substrate conditions. PLB-based systems further improved efficiency, reaching 89% PLB induction, 400% secondary PLB proliferation, 98% rooting, and full survival after acclimation, which makes them especially suitable for large-scale factory seedling production. More recent

protocols for tetraploid or floral-bud-derived materials show that standardized propagation can also be linked to elite-germplasm utilization, with high shoot induction, rooting, and long-term greenhouse survival (Zhang et al., 2025; Luan et al., 2026).

Standardization must then extend from nursery protocols to cultivation management, environmental control, and quality criteria. Industry reviews identify the lag in variety breeding, insufficient quality systems, and weak product innovation as central bottlenecks, so production standards should define improved-variety propagation, substrate and greenhouse management, harmless pest control, and dynamic monitoring from seedling to harvest (Hong et al., 2016). Practical cultivation studies already outline the core variables that can be standardized, including ecological conditions, greenhouse location, seedbed construction, fertilizer and water management, and harvest management. Evidence also supports standardizing light and substrate regimes, because blue light improves biomass, flavonoids, and polysaccharides, while suitable mixed substrates and high humidity favor transplant survival and plant quality (Ye et al., 2020). In practice, a standardized production system should integrate elite germplasm, rapid propagation, controlled environment cultivation, and multi-index quality evaluation so that cultivated material is both consistent and traceable (Wang et al., 2022; Zhang et al., 2025).

5.2 Coordinated development of resource conservation and industry of *Anoectochilus roxburghii*

The coordinated development of conservation and industry is necessary because *A. roxburghii* has high medicinal and economic value, but its wild populations have been severely depleted by over-collection, habitat loss, slow growth, and low natural regeneration. Artificial cultivation is therefore not only an industrial strategy but also a conservation substitute that reduces harvesting pressure on wild populations (Wang et al., 2018; Wang et al., 2022). The conservation side should combine in situ protection of stable habitats with ex situ germplasm preservation, especially for threatened peripheral populations and elite local resources. Distribution modeling further suggests that future cultivation bases can be planned in newly suitable northern regions to meet demand while reducing pressure on vulnerable southern wild populations (Hou et al., 2025).

Industrial development should be coupled to conservation through germplasm protection, regional planning, and wild-imitated cultivation. Earlier resource surveys already argued that preservation and rational exploitation must be considered together, rather than as separate agendas. Wild-imitated cultivation is especially promising because it shifts the endophytic community toward that of wild-tending plants and is associated with higher kinsenoside content, which helps align cultivated material with wild medicinal quality. Beneficial microbial strategies support the same conservation-industry linkage: endophytic fungi and *Bacillus velezensis* strains increase biomass and active compounds, which can raise cultivated yield and quality without increasing collection pressure on wild plants (Ye et al., 2020; Hou et al., 2025). The most sustainable pathway is therefore a coordinated model in which protected germplasm resources feed standardized artificial cultivation, and cultivation success in turn lowers incentives for destructive wild harvesting (Hong et al., 2016).

5.3 Extension of the *Anoectochilus roxburghii* industrial chain and high-value utilization

The industrial chain of *A. roxburghii* can be extended because the species is not only a medicinal plant but also a functional food and ornamental resource with diverse bioactive constituents and broad pharmacological relevance. It is already used in foods, soups, teas, beverages, and several dosage forms, and clinical or quasi-clinical applications have been reported for diabetes, hepatitis B, hyperuricemia, cough-variant asthma, and other conditions (Ye et al., 2020; Gam et al., 2020). These features support downstream diversification into health foods, botanical beverages, standardized extracts, and pharmaceutical intermediates rather than reliance on crude fresh-herb sales alone (Wang et al., 2018; Hou et al., 2025). PLBs and rhizome cultures also offer alternative production units for kinsenoside and polysaccharides, which creates opportunities for ingredient-oriented processing rather than depending exclusively on whole-plant harvests (Wang et al., 2022; Luan et al., 2026).

High-value utilization also depends on strengthening the midstream processing segment and building stronger product innovation capacity. Patent analysis indicates that current research and development already focuses on pharmaceutical preparations, food uses, and downstream products such as anti-inflammatory and anti-infective

applications, while also pointing to the need for extraction and purification equipment in the midstream segment. The same analysis suggests further expansion into medical, cosmetic, and oral-care products, including immune regulation, skin repair, whitening, and tooth-protection applications. Because current industry assessments still identify weak innovation capability, low brand competence, and limited market cognition, future industrial upgrading should combine standardized raw-material supply with deep processing, brand development, and diversified consumer products (Hong et al., 2016). For *A. roxburghii*, the most viable industrial development pathway is therefore to use artificial replacement cultivation as the upstream base, and then extend toward high-value extracts, functional foods, pharmaceutical products, and other differentiated applications that increase output value while supporting conservation.

6 Problems and Optimization Strategies

6.1 Utilization efficiency of superior germplasm resources of *Anoectochilus roxburghii* still needs improvement

Current artificial replacement cultivation has reduced dependence on wild resources, but the efficient use of elite germplasm remains limited by slow variety breeding, insufficient protection of breeding resources, and weak integration between germplasm evaluation and industrial propagation (Wei et al., 2022). Existing evidence shows that polyploid breeding is a promising route to superior germplasm utilization, because tetraploid plants generally show higher major secondary metabolite production, stronger photosynthetic performance, larger roots and stomata, and better adaptation to unstable environments than diploid materials (Su et al., 2017; Huang et al., 2022; Zhang et al., 2025). Artificial induction methods have already provided a practical basis for this direction: mutagenic tetraploid breeding reported frequencies above 75% and improved medicinal yield, while optimized in vitro propagation protocols for tetraploid materials achieved high shoot induction, rooting, and transplant survival, making elite-line expansion technically feasible (Zhang et al., 2025). At the same time, utilization efficiency is still constrained by incomplete molecular characterization of germplasm, although the recent chromosome-level autotetraploid genome now offers a reference for functional genomics, marker development, and precise molecular breeding (Fang et al., 2025).

Optimization should therefore focus on building a coordinated “collection-evaluation-breeding-propagation” system for elite germplasm. First, wild, local, and cultivated resources should be systematically collected and conserved, while superior chemotypes and stress-tolerant lines are identified through combined phenotypic, phytochemical, and molecular screening (Ye et al., 2020). Second, molecular breeding should be accelerated by using genomic resources and drought-resistance candidate genes such as the ArWRKY57-ArWRKY70-ArLEA5 module to develop elite cultivars with both stable quality and stronger environmental resilience (Jiang et al., 2025; Fang et al., 2025). Third, rapid propagation systems should be matched to elite germplasm deployment, including PLB-based regeneration, nodal culture, and tetraploid micropropagation, so that breeding gains can be translated quickly into industrial seedling supply (Zhang et al., 2025). In short, the key problem is not the absence of superior germplasm, but the low efficiency with which conserved, identified, bred, and propagated elite materials are converted into standardized cultivation resources (Su et al., 2017; Huang et al., 2022).

6.2 Quality stability of artificially cultivated *Anoectochilus roxburghii* still needs enhancement

The main quality problem in cultivated *A. roxburghii* is that active constituents vary substantially with cultivation mode, light regime, microbial association, habitat conditions, harvest stage, and post-harvest handling, so cultivated material often lacks the consistency required for medicinal use (Luo et al., 2025). Environmental regulation is especially important: blue light improves growth, flavonoids, and polysaccharides, red-blue combinations can markedly increase phenolic acids, flavonoids, and kinsenoside, and optimized orthogonal light protocols differentially favor polysaccharides or flavonoids depending on light intensity, red:blue ratio, and photoperiod (Chen et al., 2021; Luo et al., 2025). Biological regulation is similarly influential, because wild-imitated cultivation shifts the endophytic community toward wild-type structure and is associated with higher kinsenoside, while endophytic fungi, mycorrhizal fungi, AM fungi, and beneficial bacteria all improve biomass and promote accumulation of flavonoids, polysaccharides, or kinsenoside (Ye et al., 2020; Zhang et al.,

2020; Gu et al., 2025). Even after harvest, active compounds continue to change: polysaccharides, kinsenoside, and flavonoids all decline during storage, vacuum packaging better preserves polysaccharides and flavonoids, and kinsenoside degradation is influenced more by endogenous enzymes than packaging method (Wei et al., 2022).

Improving quality stability requires shifting from simple yield-oriented cultivation to process-oriented quality regulation. Cultivation standards should define target light formulas, substrate composition, humidity, temperature, and culture duration for different production objectives, since both bioreactor rhizome culture and greenhouse systems show clear optimum windows for metabolite accumulation (Jin et al., 2017; Chen et al., 2021). Microbial management should become part of routine cultivation, with prioritized use of beneficial endophytes, mycorrhizae, and AM fungi that simultaneously improve growth, quality, and disease resistance (Ye et al., 2020; Zhang et al., 2020; Gu et al., 2025). Harvest timing and elicitation strategies should also be optimized, because kinsenoside accumulation peaks at defined developmental stages and can be significantly enhanced by yeast extract, salicylic acid, or methyl jasmonate under controlled rhizome culture conditions (Jin et al., 2018; Luo et al., 2018). Finally, post-harvest drying, packaging, and storage must be standardized together with multi-component chemical monitoring, so that quality stability is controlled across the full chain rather than judged only at harvest (Wei et al., 2022).

6.3 The standardized industrial system of *Anoectochilus roxburghii* still needs improvement

Although artificial cultivation has supported expanding production, the industry still lacks a unified system linking germplasm, seedling production, cultivation management, quality evaluation, processing, storage, and downstream product development (Su et al., 2017; Wei et al., 2022). One major weakness is the absence of consistent quality standards across regions and products, compounded by species confusion and adulteration in the market because morphologically similar *Anoectochilus*, *Goodyera*, and *Ludisia* materials are sometimes used as substitutes (Ye et al., 2020; Fang et al., 2025). Another weakness is that industrial production often emphasizes planting scale more than chain integration, even though research already points to the importance of greenhouse management, harmless pest control, controlled environmental regulation, and post-harvest quality preservation (Zhang et al., 2020; Chen et al., 2021). The gap is therefore systemic: production technologies exist, but they are not yet assembled into a unified industrial specification from upstream breeding to midstream processing and downstream high-value utilization (Ye et al., 2020).

The optimization pathway is to build a full-chain standardized industrial system centered on traceability, quality markers, and coordinated industrial upgrading. Upstream, authenticated elite germplasm should be linked to standardized propagation and protected breeding-resource management, using DNA barcoding, chemotype classification, and molecular markers to prevent varietal confusion and improve source control (Ye et al., 2020; Fang et al., 2025). Midstream, production should be standardized through facility cultivation protocols, microbial-assisted quality enhancement, optimized drying and storage methods, and process specifications for extraction and purification of active ingredients (Ye et al., 2020; Gu et al., 2025). Downstream, the industry should expand from crude medicinal material sales toward functional foods, oral liquids, cosmetics, and pharmaceutical preparations, while using multi-component analytical tools to define stable Q-markers and improve product consistency (Wang et al., 2018; Zhang et al., 2020). Overall, the standardized industrial system of *A. roxburghii* still needs improvement because the next stage of artificial replacement cultivation is not simply more planting, but tighter integration of breeding, cultivation, quality control, processing, and high-value product development (Wei et al., 2022).

7 Conclusion and Prospects

Conservation of wild *Anoectochilus roxburghii* resources is the foundation for sustainable industrial development because natural populations have been severely reduced by over-collection, habitat loss, slow growth, low natural propagation, and narrow ecological requirements. Its threatened status is reflected in formal protection designations, including listing as a protected plant in China and recognition as vulnerable, near-threatened, or endangered in international conservation frameworks. Conservation planning now has clearer spatial guidance, because climate-based distribution modeling identifies Fujian, Guangdong, Guangxi, Yunnan, and Guizhou as stable highly suitable habitats that should be prioritized for in situ protection, while contracting peripheral

populations should be urgently targeted for ex situ germplasm collection to preserve adaptive diversity. Earlier field-resource surveys and recent metabolomic studies also show that conserving wild populations is important not only for species survival but also for maintaining ecological, chemical, and regional diversity that underpins future breeding, quality evaluation, and rational utilization.

Artificial replacement cultivation of *A. roxburghii* is the core pathway for relieving resource pressure because market supply can no longer rely on wild harvesting, and artificial cultivation already serves as the main source of propagated material. Rapid propagation systems now provide a practical basis for large-scale replacement, including nodal culture with 91.67% shoot formation, 93.33% rooting, and 90.2% transplant survival, PLB systems with 89% induction, 400% secondary proliferation, 98% rooting, and full acclimatization survival, and tetraploid propagation systems that support both germplasm conservation and commercial multiplication. Beyond simple yield replacement, wild-imitated cultivation, under-forest cultivation, and karst-forest imitation-wild systems provide additional routes for reducing pressure on wild resources while improving medicinal continuity and ecological compatibility. Climate projections further suggest that establishing future cultivation bases in newly suitable northern regions such as Hubei, Anhui, and southern Henan could simultaneously meet market demand and reduce harvesting pressure on vulnerable southern wild populations.

Scientific and technological innovation will promote *A. roxburghii* resource conservation and high-quality industrial development because the main remaining bottlenecks are low breeding efficiency, unstable cultivated quality, weak quality systems, and limited industrial innovation capacity. Innovation is already improving propagation efficiency and product quality through optimized media, light regulation, elite tetraploid propagation, and alternative production systems such as rhizome bioreactors and PLB-based kinsenoside production. Molecular and omics tools are expanding the breeding and management toolbox, including DNA barcode identification, transcriptome resources, a high-quality reference genome, drought-tolerance gene modules, and metabolome-transcriptome analyses showing that red-blue light can strongly promote functional metabolite biosynthesis. At the cultivation end, microbial engineering, endophyte management, and environmentally controlled production can improve biomass, disease resistance, and active-ingredient accumulation, while industrial upgrading should extend toward standardized extraction, purification, quality markers, and diversified products in medicine, health food, and cosmetics.

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Review and Progress

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Evaluation of the Effects of Harvest Period and Drying Methods on the Quality of Hangbaiju

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Abstract Hangbaiju, an important medicinal and edible plant in Zhejiang Province, has value in tea, food, and medicinal product development, and its quality is jointly affected by harvest period and post-harvest drying methods. This study explored the effects of harvest period and drying methods on the formation and preservation of Hangbaiju quality, with a focus on analyzing the effects of different flowering stages on flavonoids, phenolic acids, volatile compounds, antioxidant activity, and commercial appearance quality. It also compared the differences among sun drying, shade drying, hot-air drying, microwave-assisted drying, pulsed vacuum drying, and vacuum freeze-drying in terms of active component retention, color preservation, aroma maintenance, and processing efficiency. The results showed that the period from the bud stage to the Taiju stage is generally favorable for the accumulation of functional components such as chlorogenic acid, total flavonoids, luteolin-7-O-glucoside, and 3,5-O-dicaffeoylquinic acid, whereas the full flowering stage has certain advantages in flower integrity, yield, and commercial maturity. In terms of drying methods, moderate or staged thermal treatment, microwave-hot air combined drying, infrared-assisted drying, and pulsed vacuum drying help improve quality stability, while shade drying also shows advantages in preserving some active components and maintaining appearance quality. Overall, the quality improvement of Hangbaiju should be based on suitable harvest windows, matched drying processes, and a multi-indicator comprehensive evaluation system, thereby promoting the transformation of Hangbaiju harvest and processing from experience-based management toward standardization, precision, and quality-oriented development.

Keywords Hangbaiju; Harvest period; Drying method; Quality evaluation; Active components

1 Introduction

Hangbaiju, a cultivar of *Chrysanthemum morifolium* widely produced in Zhejiang, China, is an important traditional medicinal and edible plant that has long been used in tea, food, and health-related products. Chrysanthemum has been cultivated in China for tea and food use for more than two thousand years, and the dried capitulum is commonly consumed both as a traditional Chinese medicine and as a functional food material (Yuan et al., 2015; Ouyang et al., 2022). In Hangbaiju specifically, the capitulum is rich in flavones and caffeoylquinic acids, which are regarded as the principal active substances and are associated with anti-inflammatory, antibacterial, antioxidant, and antidiabetic activities (Lu et al., 2024). Hangbaiju is also favored by consumers because of its disease-preventing and health-improving functions, and both its flowers and leaves show notable antioxidant, antibacterial, antiviral, anti-inflammatory, antitumor, and immunomodulatory activities (Liu et al., 2025). Beyond medicinal use, chrysanthemum products are valued for flavor, aroma, and sensory quality, which supports their role in health teas and other edible applications (Xu et al., 2022a). Because Hangbaiju quality directly determines its medicinal efficacy, edible value, and market competitiveness, establishing scientific quality evaluation criteria is essential. Current studies show that relying only on geographical origin or traditional experience can lead to biased quality judgment, whereas comprehensive evaluation based on chromatographic fingerprints, bioactive compounds, and bioactivity provides a more reliable basis for quality control (Lu et al., 2022). Consistent with this, flavones and caffeoylquinic acids are not only major active ingredients but also important indicators for evaluating Hangbaiju quality, while compounds such as chlorogenic acid, 3,5-O-dicaffeoylquinic acid, luteolin, and related flavonoids repeatedly emerge as key discriminatory or quality-marker substances across chrysanthemum cultivars.

The quality of Hangbaiju is not static, but is formed dynamically during flower development and is strongly influenced by harvest period. Developmental-stage studies on Hangbaiju have shown that total flavonoid content is higher in the early stages S1 and S2, while total polyphenol content rises first and then declines, peaking at S2, indicating that major active constituents accumulate preferentially before full senescence (Lu et al., 2024). More broadly in medicinal chrysanthemum, flavonoid and chlorogenic acid contents differ significantly across floral development stages and reach their highest values at the bud stage, while comprehensive analysis suggests that the best harvest stage for simultaneously obtaining relatively high active and nutritional ingredients lies between the bud and young flower stages. Similar patterns have been reported in other chrysanthemum materials, where most differential flavonoids and phenolic acids were higher at the initial or full-bloom stages than at late stages, supporting the idea that optimal harvest windows are closely tied to metabolite accumulation patterns (Yang et al., 2024). Even when antioxidant activity does not always shift in parallel, harvest stage still significantly affects moisture, phenolics, flavonoids, anthocyanins, and carotenoids, and its interaction with genotype can further shape functional quality (Julianti et al., 2026). In addition, morphological traits may reflect chemical quality: in a Hangbaiju-related subtype, smaller inflorescence diameter was associated with higher chlorogenic acid and 3,5-O-dicaffeoylquinic acid contents, suggesting that external appearance can have value when linked with internal markers in quality grading (Yang et al., 2025). Together, these findings indicate that harvest timing is a key determinant of the accumulation of active substances and therefore a central factor in the quality formation of Hangbaiju.

After harvest, drying is the critical processing step that further determines whether the intrinsic quality of Hangbaiju can be preserved, enhanced, or degraded. Drying is widely used to prolong shelf life, improve storability, and support the processing of chrysanthemum into teas and medicinal products, but it also alters chemical composition, color, aroma, microstructure, and bioactivity (Xu et al., 2022a; Wang et al., 2024). Multiple studies show that drying method significantly affects bioactive constituents and antioxidant properties in chrysanthemum flower heads (Yuan et al., 2015; Shi et al., 2017). In medicinal chrysanthemum, appropriate oven drying has been recommended at about 55~65 °C, while for specific cultivars oven-drying at 60 °C or 70 °C produced favorable outcomes, indicating that suitable temperature ranges can improve the retention of active ingredients (Shi et al., 2017). Drying technology also matters beyond simple temperature control: combined infrared and hot-air drying increased chlorogenic acid, luteolin, total phenolics, and total flavonoids, and infrared-assisted methods strongly affected aroma retention and color behavior. Pulsed vacuum drying preserved total phenolics and flavonoids, enhanced antioxidant activity, and favored volatile retention under lower temperature and pulsed ratio conditions, while vacuum drying more generally was reported to retain antioxidant components in a shorter time (Sun et al., 2021; Lu et al., 2020). Microwave-assisted and combined pretreatments can also improve quality by inhibiting enzymatic browning and preserving biologically active compounds, although excessive heat or prolonged treatment may accelerate oxidation and degradation of flavonoids and other heat-sensitive constituents (Wang et al., 2019; Xu et al., 2022a; Miao et al., 2026). Recent work further shows that kill-green pretreatment can shorten subsequent drying time and improve color, phenolics, amino acids, volatiles, and sensory acceptance, emphasizing that processing sequence as well as drying mode contributes to final quality (Xu et al., 2024).

This study will investigate the effects of harvest period and drying methods on the formation and preservation of Hangbaiju quality, with the aim of further clarifying the major characteristics of quality changes in Hangbaiju under different harvest stages and processing conditions. The quality of Hangbaiju is not determined by a single factor, but results from the coordinated effects of multiple chemical markers, antioxidant-related properties, sensory traits, and morphological characteristics; meanwhile, floral developmental stage and post-harvest processing can both significantly alter these quality indicators. However, current studies often examine harvest stage or drying technology separately, and some studies focus mainly on other chrysanthemum cultivars. Therefore, a comprehensive evaluation of the combined effects of harvest period and drying methods on Hangbaiju remains necessary. By comparing changes in key active constituents, nutritional and functional indicators, and related quality traits of Hangbaiju under different harvest periods and drying methods, this study

analyzes the patterns of quality formation and preservation, screens harvest and processing conditions suitable for the production of high-quality raw materials, and provides a reference for optimizing field harvest decisions, improving post-harvest processing parameters, enhancing product quality consistency, and promoting the standardized development of the Hangbaiju industry.

2 Quality Evaluation Indicator System for Hangbaiju

2.1 Appearance and commercial quality indicators of Hangbaiju

Appearance and commercial quality are the most direct indicators in the primary grading of Hangbaiju, because consumers and traders usually judge flower products first by inflorescence integrity, size, color, and external uniformity. Chrysanthemum flower color and luster are recognized consumer-facing quality traits, while petal color and shape also affect preference and market acceptance (Xu et al., 2022a; Wei et al., 2024). At the same time, appearance alone is not a reliable proxy for intrinsic quality, because different quality grades may show no obvious difference in smell or visible traits, and market practices such as sulfur fumigation can artificially improve appearance while reducing antioxidant activity and bioactive constituents (Wei et al., 2024).

For Hangbaiju-related materials, inflorescence morphology has practical grading value when linked with chemical markers. In Fubaiju, a subvariety introduced from Hangbaiju, inflorescence diameter was negatively correlated with chlorogenic acid and 3,5-O-dicaffeoylquinic acid, and first-grade samples had significantly smaller diameters and higher polyphenol contents (Yang et al., 2025). Studies using hyperspectral imaging further show that exterior morphology can be fused with spectral information to predict total flavonoids and chlorogenic acids with high accuracy, supporting the use of image-based appearance indexes as rapid commercial evaluation tools rather than purely subjective observations (Wei et al., 2024).

2.2 Active components and physicochemical indicators of Hangbaiju

Active components and physicochemical indexes are the core of scientific Hangbaiju quality evaluation, because the nutritive and commercial values of chrysanthemum tea depend directly on phytochemical composition. Flavones and caffeoylquinic acids are the main active ingredients of Hangbaiju and are explicitly identified as important indicators for evaluating its quality (Lu et al., 2024). Across chrysanthemum cultivars, chlorogenic acid, luteolin, apigenin-7-glucoside, cryptochlorogenic acid, and isochlorogenic acid C contribute strongly to varietal discrimination, while spectrum-effect analysis has further identified chlorogenic acid, 3,5-O-dicaffeoylquinic acid, 4,5-O-dicaffeoylquinic acid, and kaempferol-3-O-rutinoside as candidate Q-markers for quality evaluation (Ouyang et al., 2022; Lu et al., 2022).

The physicochemical indicator system should therefore include total flavonoids, total phenolics, chlorogenic acid and related CQA derivatives, moisture-related drying sufficiency, antioxidant activity, and selected nutritional indexes such as amino acids, soluble sugars, and vitamin C. Developmental studies in Hangbaiju showed that total flavonoids were higher at S1-S2 and total polyphenols peaked at S2, while other chrysanthemum studies found that flavonoid and chlorogenic acid contents were highest at the bud stage or between the bud and young flower stages (Lu et al., 2024). Drying also reshapes these indexes: oven drying at suitable temperatures, microwave-hot air drying, pulsed vacuum drying, and infrared-assisted drying each improved retention of different phenolics or antioxidant properties, showing that physicochemical evaluation must be tied to post-harvest processing conditions rather than measured as a fixed inherent trait (Wang et al., 2019; Sun et al., 2021; Xu et al., 2022a).

2.3 Sensory quality and safety indicators of Hangbaiju

Sensory quality and safety indicators form the third essential dimension of Hangbaiju evaluation because chrysanthemum is both a medicinal and edible material used in tea and food applications (Xu et al., 2022a). Sensory quality includes aroma, taste, color stability, and overall acceptance after processing. Different drying strategies dramatically change aroma profiles, and infrared-hot air drying produced the highest volatile concentration, while kill-green pretreatment with high-humidity air impingement improved color and retained more phenolics, amino acids, volatile compounds, and higher sensory acceptance than steam kill-green (Xu et al., 2024).

Safety evaluation should emphasize authenticity, processing cleanliness, and the stability of active compounds during storage and heating. Appearance-based adulteration is a real concern in chrysanthemum tea, because sulfur fumigation can counterfeit quality while damaging antioxidant properties and causing loss of bioactive compounds (Wei et al., 2024). In addition, chrysanthemum flavonoids are sensitive to light, oxygen, high temperature, and extreme pH, so storage and processing stability should be treated as part of product safety and quality assurance (Miao et al., 2026). Functional verification can also assist sensory-safety grading: first-grade Fubaiju showed stronger saltiness and astringency by electronic tongue and stronger antibacterial effects against *Escherichia coli* and *Staphylococcus aureus*, indicating that sensory discrimination and biological activity can serve as auxiliary indexes in Hangbaiju quality evaluation (Yang et al., 2025).

3 Effects of Harvest Period on the Quality of Hangbaiju

3.1 Effects of harvesting at the bud stage and early flowering stage on the quality of Hangbaiju

Harvesting Hangbaiju at the bud stage and early flowering stage generally favors the accumulation of key medicinal constituents, especially flavonoids and chlorogenic-acid-related compounds. In Hangbaiju capitula, total flavonoids were higher at S1 and S2 than at later stages, and total polyphenols rose first and then declined, peaking at S2 (Lu et al., 2024). HPLC-based analysis of four Hangbaiju products likewise showed that total flavonoids and chlorogenic acid reached their maximum at the Hualei stage, with Taiju ranking next. In medicinal chrysanthemum more broadly, flavonoid and chlorogenic acid contents were highest at the bud stage, and comprehensive evaluation placed the best harvest window between the bud and young-flower stages when both active and nutritional ingredients were considered (Ma et al., 2016). Untargeted and targeted metabolomics in *Chrysanthemum indicum* also showed that most varying flavonoids and phenolic acids were higher at the initial stage than at the eventual stage, supporting the value of early harvest for preserving active compounds (Yang et al., 2024).

Early harvest also appears advantageous for commercial differentiation and antioxidant potential, although the exact optimum depends on which compounds are prioritized. Taiju, defined as flower heads with opened ray florets but closed tubular florets and harvested earlier than Duoju, showed higher caffeoylquinic acids and stronger antioxidant activities than the later-harvested Duoju samples (Gong et al., 2019). In Hangbaiju harvested at different collection periods, metabolomics identified distinct volatile and flavonoid markers between Taiju and Duoju, and in Taiju most of the differential flavor volatiles and five of six flavonoid markers showed upward trends, indicating that earlier harvest contributes to a unique flavor and efficacy profile (Yang et al., 2022). A related grading study on Fubaiju, a Hangbaiju subvariety, found that smaller inflorescence diameter was associated with higher chlorogenic acid and 3,5-O-dicaffeoylquinic acid, and these trends were consistent with changes across the chrysanthemum picking period (Yang et al., 2025). Together, these findings indicate that bud-stage to early-flowering harvest is usually more favorable when the goal is to maximize polyphenol-rich medicinal quality rather than flower size or yield.

3.2 Effects of harvesting at the full flowering stage on the quality of Hangbaiju

Harvesting at the full flowering stage often provides a balanced quality profile, especially when producers must weigh active constituents against morphology, harvest efficiency, and marketable output. Developmental classification work in edible Hangju separates blooming into five stages and places harvest standards around stages 4 and 5, indicating that practical harvest commonly occurs near full opening rather than at the earliest bud stages (Liu et al., 2025). A cultivar study comparing half-bloom and full-bloom flowers found that harvest stage significantly affected moisture, total phenolics, total flavonoids, anthocyanins, and β -carotene, even though antioxidant activity did not differ significantly overall (Julianti et al., 2026). The same study also showed that harvest stage interacted with cultivar to alter phenolics, anthocyanins, β -carotene, and antioxidant activity, which suggests that full-bloom performance cannot be judged independently of genotype (Julianti et al., 2026).

For Hangbaiju specifically, full bloom appears to be a compromise stage rather than the peak for most medicinal markers. The 2011 Hangbaiju harvesting-stage study showed that delaying harvest increased yield markedly, but the highest total flavonoids and chlorogenic acid occurred earlier than full flowering, while luteolin-7-O-glucoside

and 3,5-O-dicaffeoylquinic acid peaked at the Taiju stage rather than the latest stage. In Fubaiju, literature summarized within the grading study noted that chlorogenic acid and 3,5-O-DCQA were higher at initial bloom than at full bloom, while luteolin-7-O-glucoside varied little across the Hangbaiju flowering period (Yang et al., 2025). Even so, full bloom can remain commercially acceptable because flower heads are morphologically complete and easier to standardize, and moderate environmental conditions can concentrate harvest timing and improve flower yield near marketable stages (Liu et al., 2025). Therefore, full-flowering harvest is suitable when a balance is needed between visual maturity, operational convenience, and acceptable internal quality, but it usually does not maximize the full spectrum of active ingredients.

3.3 Effects of harvesting at the late flowering stage on the quality of Hangbaiju

Harvesting Hangbaiju at the late flowering stage generally reduces core bioactive quality, especially for flavonoids, chlorogenic acid derivatives, and some antioxidant-related markers. In Hangbaiju metabolomic analysis, flavones and caffeoylquinic acids accumulated at higher levels in S1 and S2 and then gradually declined from S3 to S5, with S5 corresponding to the stage when the outer whorl of ray florets began to decay (Figure 1) (Lu et al., 2024). The broader chrysanthemum developmental study under UV-B likewise showed that flavonoid and chlorogenic acid contents were highest at the bud stage rather than later stages, while soluble sugar, amino acid, and vitamin C increased as flowers developed, demonstrating that late harvest shifts quality away from medicinal phenolics toward some nutritional traits (Ma et al., 2016). In *Chrysanthemum indicum*, eventual-stage samples differed clearly from initial and full-bloom samples, and most varying constituents were lower at the eventual stage than at initial or full bloom (Yang et al., 2024).

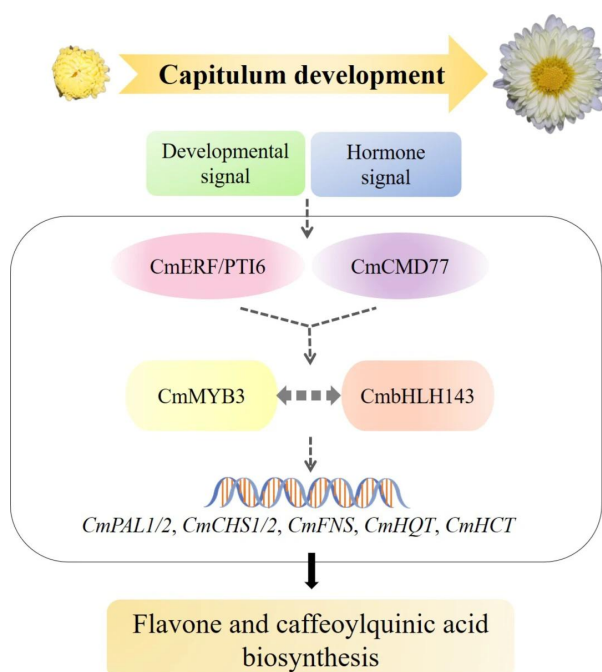


Figure 1 Mechanisms of flavone and CQA accumulation during the development of chrysanthemum capitulum (Adopted from Lu et al., 2024)

Image caption: The dotted lines represent the predicted interaction relationship or hypothetical regulatory network (Adopted from Lu et al., 2024)

Late harvest may still increase flower yield and opening degree, but these gains tend to come with weaker medicinal and sensory advantages. The Hangbaiju stage-comparison study found that yield increased obviously as collection time was deferred, yet overall evaluation still identified the Taiju stage as the best harvest time when functional constituents, yield, and timing were considered together. Comparison of commercial Hangbaiju types also showed that the later-harvested Duoju, collected when both ray and tubular florets were fully opened, had lower caffeoylquinic acids and weaker antioxidant activities than the earlier-harvested Taiju (Gong et al., 2019). Because harvest time also affects inflorescence diameter, and larger-diameter samples are associated with lower

chlorogenic acid and 3,5-O-dicaffeoylquinic acid in Fubaiju, excessive delay in harvest likely improves external fullness at the expense of internal quality markers (Yang et al., 2025). Overall, late flowering harvest is less favorable for high-quality Hangbaiju if the evaluation emphasizes medicinal actives, antioxidant capacity, and premium-grade commercial quality rather than yield alone.

4 Effects of Drying Methods on the Quality of Hangbaiju

4.1 Effects of sun drying and shade drying on the quality of Hangbaiju

Sun drying and shade drying are traditional methods for processing chrysanthemum, but their effects on Hangbaiju quality are not identical. Studies comparing sun-dried, shade-dried, and oven-dried chrysanthemum flower heads showed that drying process significantly altered both bioactive constituent contents and antioxidant properties, indicating that natural drying cannot be regarded as a neutral preservation step. Metabolomic comparison of shade drying and heat drying further showed that the two methods produced clear differences in flavonoids, phenolic acids, and terpenoids, and that the levels of key indicator compounds and their precursors also changed significantly between treatments. In that comparison, most flavonoids, major phenolic acids, terpenoids, and carbohydrates were higher after shade drying than after heat drying, suggesting that slower, milder dehydration is more favorable for preserving many medicinally relevant components (Chen et al., 2024).

However, the advantages of natural drying are method-specific and must be weighed against efficiency and quality stability. One study found that nature and vacuum drying were both beneficial for increasing phenolic contents and antioxidant capacities in chrysanthemum flowers, indicating that low-stress dehydration can help preserve functional quality (Lu et al., 2020). Another study reported that total phenols and total flavonoids in infrared-dried chrysanthemums were significantly higher than in shade-dried ones, which implies that shade drying does not necessarily maximize active-component retention when compared with improved low-thermal technologies (Xu et al., 2022a). More broadly, direct sun exposure in plant materials is often associated with higher losses of light- and heat-sensitive pigments, because open-air drying exposes tissues to oxygen, radiation, and prolonged dehydration time (Belwal et al., 2022). Therefore, for Hangbaiju, shade drying is generally preferable to sun drying for preserving internal quality, but its long drying cycle and weaker process control limit its suitability for standardized, large-scale production.

4.2 Effects of hot-air drying on the quality of Hangbaiju

Hot-air drying is the most common industrial method for chrysanthemum because the equipment is simple and the drying rate is fast, but product quality depends strongly on temperature control. Chrysanthemum studies consistently show that increasing hot-air temperature shortens drying time and increases drying rate and moisture diffusivity (Xu et al., 2022a). At the same time, prolonged exposure to hot aerobic conditions can cause losses of bioactive and volatile substances and can also induce browning and petal curling, so faster drying does not automatically mean better quality. In chrysanthemum flowers, conventional hot-air drying produced the lowest total flavonoid values in one direct comparison, which was attributed to reactions between flavonoids and oxidase during drying (Wang et al., 2019).

The best hot-air conditions therefore appear to be moderate rather than extreme. For flower heads harvested at different developmental stages, appropriate oven drying was recommended at 60 °C for Xiaobaiju and 70 °C for Taiju. Another study concluded that an oven temperature of about 55~65 °C could simultaneously maintain relatively high active and nutritional ingredient contents (Shi et al., 2017). Evidence from pulsed-vacuum and related low-oxygen drying also supports the same general principle: lower temperature or staged heating better protects color, chlorogenic acid, luteolin, total phenolics, total flavonoids, and antioxidant capacity, whereas excessive temperature increases thermal degradation despite higher efficiency (Figure 2) (Xu et al., 2022a). Variable-temperature drying can even increase phenolic compounds, total flavonoids, and antioxidant capacity in chrysanthemum tea, showing that controlled thermal profiles can promote quality formation rather than simple degradation (Sun et al., 2026). Thus, hot-air drying is practical for Hangbaiju processing, but its final quality is governed by the coordinated control of temperature, time, and oxygen exposure.

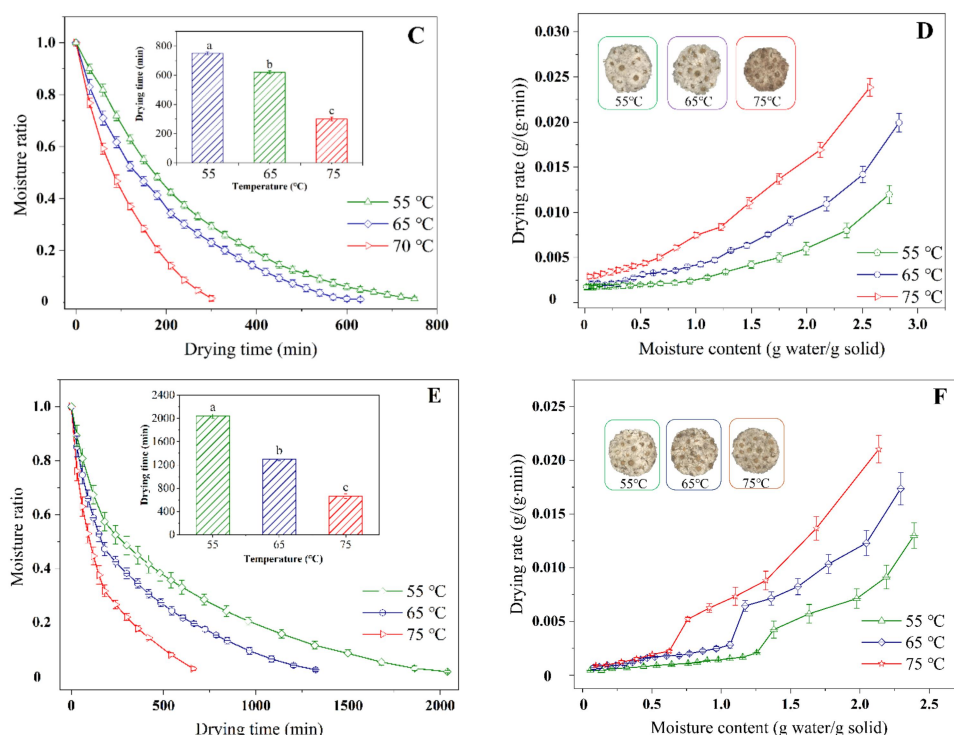


Figure 2 Moisture ratio (A,C,E) and drying rate (B,D,F) of chrysanthemum cakes under different drying methods (Adopted from Xu et al., 2022a)

Image caption: (A, B): Hot air drying (HAD); (C, D): Combined infrared and hot air drying (IR-HAD); (E, F): Sequential IR-HAD and HAD (IR-HAD+HAD), different lowercase letters reveal significant differences ($p < 0.05$) (Adopted from Xu et al., 2022a)

4.3 Effects of microwave drying and vacuum freeze-drying on the quality of Hangbaiju

Microwave drying and vacuum freeze-drying represent two contrasting technical routes for improving Hangbaiju quality: one emphasizes rapid dehydration and enzyme inactivation, and the other emphasizes low-temperature preservation. In medicinal chrysanthemum, flavone and chlorogenic acid contents increased significantly with increasing microwave power, although amino acid content decreased significantly under microwave treatment (Shi et al., 2017). Considering efficiency and energy consumption, microwave drying was identified as the most suitable method among four tested methods for retaining higher flavone, vitamin C, and soluble sugar contents. In a direct comparison of drying methods, microwave treatment for 30 s combined with 75 °C hot-air drying was the most effective at preserving biologically active compounds and produced higher antioxidant capacity and stronger acetylcholinesterase inhibition (Wang et al., 2019). Another kinetics study similarly found that mild microwave-assisted hot-air treatment retained higher total phenolics, total flavonoids, and seven monomeric compounds with only limited conformational change (Wang et al., 2018).

Vacuum freeze-drying generally performs best for preserving physical structure, appearance, and many bioactive compounds, but its cost and drying time are major drawbacks. Freeze-drying improves the bioactive composition and physical structure of chrysanthemum products, although it is often considered economically unrealistic for seasonal herbs because of high energy consumption and equipment investment (Xu et al., 2022a). In Taihang chrysanthemum, freeze-drying maintained phenylpropanoid-rich bioactive compounds, gave superior antioxidant activity, and also supported stronger antibacterial and pancreatic lipase inhibitory activities (Fan et al., 2024). Evidence from other dried floral materials points in the same direction: vacuum freeze-dried petals showed the best appearance, highest bioactive compounds and antioxidant activity, and the best sensory similarity to fresh infusions (Suo et al., 2023). Freeze-dried samples also tend to preserve lighter color and heat-sensitive carotenoids better than convective drying, although the retention of phenolics can still depend on the specific plant matrix (Chua et al., 2019). Therefore, for Hangbaiju, microwave-assisted drying is more advantageous when processing efficiency and active-component retention must be balanced, whereas vacuum freeze-drying is more suitable

when the goal is premium-grade product quality and maximum preservation of appearance and functional constituents.

5 Interactive Effects of Harvest Period and Drying Methods on the Quality of Hangbaiju

5.1 Adaptability of Hangbaiju raw materials at different maturity stages to drying methods

The adaptability of Hangbaiju raw materials at different maturity stages to drying methods is mainly reflected in their different starting compositions, tissue states, and responses to heat and dehydration. Harvest stage significantly affects the functional constituents of Hangbaiju, and the contents of total flavonoids and chlorogenic acid were highest at the Hualei stage, while luteolin-7-O-glucoside and 3,5-O-dicaffeoylquinic acid peaked at the Taiju stage. Taiju and Duoju, which are harvested at different times, also show clearly different phenolic profiles and antioxidant properties, with Taiju containing higher caffeoylquinic acids and stronger antioxidant activities than Duoju (Gong et al., 2019). Metabolomic analysis further showed that Taiju and Duoju differ in both volatile oils and flavonoids, and that most characteristic flavonoids and flavor-related volatiles increased in Taiju, indicating that early-and mid-maturity materials enter drying with different chemical bases and flavor potentials (Yang et al., 2022).

Because the raw material differs by maturity stage, the most suitable drying conditions also differ. A direct study of chrysanthemum flower heads harvested at two developmental stages found that drying process significantly affected bioactive constituents and antioxidant properties, and recommended oven drying at 60 °C for Xiaobaiju and 70 °C for Taiju. More generally, the nature of the starting material, including harvesting time, strongly influences drying dynamics and compound degradation, so drying parameters should be optimized together with raw-material condition rather than chosen independently (Belwal et al., 2022). This interaction is also consistent with process studies showing that structural state governs drying behavior: tempering reduced moisture heterogeneity between the central and marginal parts of chrysanthemum and improved subsequent drying performance, indicating that flowers at different maturity stages are likely to differ in their tolerance to the same drying route (Xu et al., 2023).

5.2 Effects of harvest period and drying conditions on the retention of active components in Hangbaiju

The retention of active components in Hangbaiju depends on the combined effect of harvest period and drying conditions, because flowers harvested at different stages begin with different dominant compounds and these compounds differ in thermal stability. In chrysanthemum flowers, phenolic acids were more stable than flavonoids during dehydration (Lu et al., 2020). This matters for Hangbaiju because early or Taiju-stage materials are richer in flavonoids, chlorogenic acid, and caffeoylquinic acids (Gong et al., 2019), so poorly controlled drying can erase the compositional advantages created by earlier harvest. Quality-marker studies also indicate that chlorogenic acid, 3,5-O-dicaffeoylquinic acid, 4,5-O-dicaffeoylquinic acid, and kaempferol-3-O-rutinoside are key compounds for chrysanthemum quality evaluation, making their retention especially important when assessing harvest-processing interactions (Lu et al., 2022).

The evidence suggests that moderate or staged drying preserves these compounds better than uncontrolled high heat, while some intensified methods can further improve retention. Conventional hot-air drying gave the lowest total flavonoid values in one comparison, whereas microwave-hot air drying for 30 s plus 75 °C hot air best preserved biologically active compounds and increased antioxidant capacity (Wang et al., 2019). In medicinal chrysanthemum, microwave drying retained higher flavone, vitamin C, and soluble sugar overall, but amino acid content fell as microwave power increased, showing that the best method depends on which quality dimension is prioritized (Shi et al., 2017). Pulsed vacuum drying and multi-stage low-oxygen drying better preserved chlorogenic acid, luteolin, total phenolics, and total flavonoids, while tempering-incorporated infrared-assisted hot-air drying further increased chlorogenic acid, luteolin, total phenolic content, total flavonoid content, antioxidant capacity, and volatile compounds (Sun et al., 2021; Xu et al., 2022a; Xu et al., 2023).

5.3 Effects of harvest-processing combinations on the comprehensive quality of Hangbaiju

The best harvest-processing combination for Hangbaiju should be judged by comprehensive quality, not by a single component, because appearance, color, aroma, antioxidant activity, and sensory acceptance often respond

differently to the same treatment. Earlier-harvested Taiju appears to provide the strongest starting point for comprehensive quality, since it balances functional constituents with yield better than Hualei, Youju, or Quanju. It also shows stronger antioxidant activity than later-harvested Duoju and carries a distinctive flavor-related metabolite profile (Gong et al., 2019; Yang et al., 2022). This makes Taiju-stage raw material especially suitable for high-quality processing, provided that the drying method protects both phenolic compounds and volatile aroma substances.

Among processing options, no single method dominates all endpoints, but several combinations stand out. Infrared-assisted hot-air drying increased chlorogenic acid, luteolin, total phenolics, total flavonoids, and volatile concentration, while sequential IR-HAD plus HAD better controlled color deterioration (Xu et al., 2022a). High-humidity air impingement kill-green for 60 s improved PPO and POD inactivation, retained more individual phenolics, sugars, amino acids, and volatiles than steam kill-green, and produced higher sensory acceptance (Xu et al., 2024). Freeze-drying maintained bioactive compounds and strong antioxidant and antibacterial activities in Taihang chrysanthemum, but other work showed that microwave-assisted or moderate-temperature oven drying can provide better efficiency or a more practical quality-cost balance (Shi et al., 2017; Fan et al., 2024). For Hangbaiju, the current evidence therefore supports a strategy in which Taiju or near-Taiju raw material is paired with a moderate, staged, or assisted drying process rather than late-harvest material combined with simple high-temperature drying.

6 Comprehensive Quality Evaluation Methods for Hangbaiju

6.1 Determination and comparison of single quality indicators of Hangbaiju

The determination and comparison of single quality indicators of Hangbaiju should begin with the targeted quantification of representative active constituents. HPLC has been used to simultaneously quantify eight bioactive compounds in chrysanthemum flower heads harvested at different stages and subjected to different drying processes, showing that single-index comparison remains the foundation of process evaluation. Near-infrared hyperspectral imaging has also shown good feasibility for the rapid prediction of luteolin and quercetin in fresh and dry Hangbaiju, indicating that instrumental screening can complement conventional wet chemistry for routine quality control (He et al., 2021). In practice, chlorogenic acid, luteolin-7-O-glucoside, 3,5-O-dicaffeoylquinic acid, flavonoids, phenolics, soluble sugar, amino acids, and vitamin C are the single indicators most often compared across harvest and drying treatments (Shi et al., 2017; Sun et al., 2021).

Single indicators should not be limited to chemical contents alone, because sensory, morphological, and functional traits also discriminate quality. Inflorescence diameter was negatively correlated with chlorogenic acid and 3,5-O-dicaffeoylquinic acid in Fubaiju, and samples with smaller diameters had higher polyphenol contents, supporting the use of appearance traits as measurable grading indexes. Electronic tongue analysis further distinguished high- and low-grade samples by stronger saltiness and astringency, while antibacterial assays confirmed better inhibitory activity in the higher-grade group (Yang et al., 2025). Antioxidant activity can also serve as a single functional endpoint, and antioxidant activity–fingerprints based on HPLC-DPPH-MS and NIR calibration have shown strong predictive ability for chrysanthemum quality assessment (Zhang et al., 2022).

6.2 Construction of a multi-indicator comprehensive evaluation model for Hangbaiju

A multi-indicator comprehensive evaluation model for Hangbaiju should integrate fingerprint similarity, multi-component quantification, biological activity, and multivariate statistics into one framework. In *Chrysanthemum morifolium*, chromatographic fingerprints of 30 flower-head samples were analyzed by similarity analysis, cluster analysis, and principal component analysis, and the common peaks accounted for major differences among samples (Lu et al., 2022). Spectrum–effect analysis further linked chemical peaks to antioxidant activity and identified chlorogenic acid, 3,5-O-dicaffeoylquinic acid, 4,5-O-dicaffeoylquinic acid, and kaempferol-3-O-rutinoside as candidate Q-markers, which provides a model for linking measurable compounds to actual efficacy. A related integrated approach in edible chrysanthemum combined HPLC, chemometrics, chromatogram–effect relationships, and bioinformatics to discover Q-markers and authenticate samples, showing that multi-indicator models can simultaneously serve quality evaluation and authenticity control (Yuan et al., 2022).

For Hangbaiju, the construction of such a model should also include weighted evaluation and classification steps. Analytic hierarchy process can be used to assign weights to different indicators, simplify complex evaluation problems, and identify the best scheme when multiple traits must be balanced (Zhao et al., 2022). In garden chrysanthemum, AHP combined with K-means clustering generated comprehensive scores and classified materials into four grades, illustrating a transferable framework for grading Hangbaiju lots or process treatments (Zhao et al., 2022). Spectroscopic chemometric models also support rapid multi-index evaluation: excitation-emission matrix fluorescence with random forest regression predicted 12 functional compounds while simultaneously authenticating geographical origin, and this “two-in-one” strategy is directly relevant to rapid Hangbaiju screening (Guo et al., 2025).

6.3 Screening of the optimal combination of harvest period and drying method for Hangbaiju

The screening of the optimal combination of harvest period and drying method for Hangbaiju should be based on comprehensive quality rather than on yield or one compound alone. Harvest stage significantly affected the functional constituents of Hangbaiju, and the contents of total flavonoids and chlorogenic acid were highest at the Hualei stage, whereas luteolin-7-O-glucoside and 3,5-O-dicaffeoylquinic acid were highest at the Taiju stage (Zhao et al., 2022). Because yield increased as collection time was delayed, the best harvest period could only be selected after balancing constituent retention against output, and that comparison favored the Taiju stage overall (Lu et al., 2020). This conclusion is consistent with metabolomic evidence showing that Taiju and Duoju have distinct volatile and flavonoid profiles, giving Taiju a different flavor and efficacy basis (Yang et al., 2022).

Drying-method screening should then be matched to the selected harvest stage. For flower heads harvested at two developmental stages, the appropriate drying process was suggested to be oven-drying at 60 °C for Xiaobaiju and 70 °C for Taiju, directly showing that maturity stage changes the optimal drying condition. Other studies indicate that microwave drying best preserved flavone, vitamin C, and soluble sugar when efficiency was considered (Shi et al., 2017), while microwave treatment for 30 s combined with 75 °C hot air was most effective at preserving biologically active compounds and antioxidant capacity (Wang et al., 2019). More advanced methods such as tempering-incorporated infrared-assisted hot-air drying and pulsed vacuum drying further improved chlorogenic acid, luteolin, total phenolics, total flavonoids, antioxidant capacity, volatile retention, or color preservation (Xu et al., 2023; Sun et al., 2021). Across specialty crops more broadly, intermediate harvest timing and drying temperatures around 30~60 °C tend to perform best, which supports the principle that Hangbaiju optimization should avoid both overly early harvest and excessive thermal stress (Pedrosa et al., 2024).

7 Existing Problems and Optimization Strategies

7.1 Non-uniform harvest standards for Hangbaiju and optimization strategies

A major problem is that Hangbaiju harvest standards remain insufficiently uniform, even though harvest stage clearly changes composition, antioxidant capacity, and product identity. In Hangbaiju, total flavonoids and chlorogenic acid peaked at the Hualei stage, while luteolin-7-O-glucoside and 3,5-O-dicaffeoylquinic acid peaked at Taiju, and overall evaluation favored Taiju when constituents, yield, and timing were considered together. Taiju and Duoju also separate clearly by phenolic profile, with Taiju showing higher caffeoylquinic acids and stronger antioxidant activities, and PCA distinguished the two groups using phenolic variables (Gong et al., 2019). Metabolomics likewise identified 11 markers that can authenticate Taiju and Duoju, indicating that harvest period creates stable chemical signatures rather than only visual differences (Yang et al., 2022).

Optimization should therefore replace rough visual or customary picking rules with maturity-indexed harvest criteria. A practical framework is to define harvest windows by inflorescence opening stage together with indicator compounds such as chlorogenic acid, luteolin-7-O-glucoside, and 3,5-O-dicaffeoylquinic acid. Diameter-based grading can help operationalize this, because smaller-diameter Fubaiju samples had higher chlorogenic acid and 3,5-O-dicaffeoylquinic acid contents and were validated by sensory and antibacterial differences (Yang et al., 2025). Since Hangbaiju quality also varies with geography, harvest time, and cultivation management, unified standards should combine maturity stage with origin and batch testing rather than rely on calendar date alone (Zhang et al., 2023).

7.2 Instability of drying process parameters for Hangbaiju and optimization strategies

A second problem is the instability of Hangbaiju drying parameters, because different methods change active compounds, antioxidant properties, color, volatiles, and energy use in different directions. Drying process significantly affected bioactive constituents and antioxidant properties in Xiaobaiju and Taiju, and the suggested optimal oven conditions already differed by harvest type: 60 °C for Xiaobaiju and 70 °C for Taiju. More broadly, medicinal chrysanthemum studies found that the best oven temperature was about 55~65 °C, while optimal microwave power, microwave time, steam kill-enzyme time, and kill-enzyme time each had different windows (Shi et al., 2017). The absence of a single best method is reinforced by metabolomics showing that shade drying and heat drying produced significantly different flavonoids, phenolic acids, and terpenoids, with most key flavonoids and phenolic acids higher after shade drying (Chen et al., 2024).

Optimization should focus on process stabilization by product type, not one uniform drying recipe. For quality retention, moderate or staged heating performs better than excessive heat: multi-stage pulsed-vacuum drying better protected color, chlorogenic acid, luteolin, total phenolics, total flavonoids, and antioxidant capacity than higher-temperature schedules (Xu et al., 2022b). Targeted process intensification can further improve consistency, because tempering-incorporated infrared-assisted hot-air drying reduced drying time and energy use while increasing chlorogenic acid, luteolin, total phenolics, total flavonoids, antioxidant capacity, and volatile number (Xu et al., 2023). Pretreatment control is also critical: high-humidity air impingement kill-green shortened subsequent drying time by up to 46.15%, improved color through faster PPO and POD inactivation, and retained more phenolics, sugars, amino acids, and volatiles than steam kill-green (Xu et al., 2024). For premium products, microwave-hot air, pulsed vacuum, or freeze-drying can be selected when the priority is bioactive retention rather than lowest cost (Wang et al., 2019; Sun et al., 2021; Fan et al., 2024).

7.3 Incomplete quality evaluation system for Hangbaiju and optimization strategies

A third problem is that the Hangbaiju quality evaluation system is still incomplete, because traditional judgment by habitat or processing history alone neglects bioactive ingredients and functional quality. A spectrum-effect study on *Chrysanthemum morifolium* concluded that evaluation strategies based mainly on habitat and processing can deviate from true quality, and identified chlorogenic acid, 3,5-O-dicaffeoylquinic acid, 4,5-O-dicaffeoylquinic acid, and kaempferol-3-O-rutinoside as candidate Q-markers. Fingerprint similarity among 30 chrysanthemum samples ranged widely, and cluster analysis could divide them into multiple classes, showing substantial heterogeneity even within commercial materials (Lu et al., 2022). Methods that integrate chemical data with sensory traits are more discriminating: electronic tongue captured overall solution characteristics, and in Fubaiju the higher-grade group showed stronger saltiness and astringency together with better antibacterial activity (Yang et al., 2025).

Optimization should build a multi-indicator comprehensive evaluation model for Hangbaiju. A strong template is the combined use of chromatographic fingerprinting, multi-component quantification, antioxidant assays, and spectrum-effect relationships to define Q-markers and rank samples (Lu et al., 2022). Weighting methods such as analytic hierarchy process, followed by clustering, can convert multiple quality dimensions into comprehensive scores and grade categories (Zhao et al., 2022). Rapid instrumental models can support implementation: near-infrared hyperspectral imaging predicted luteolin and quercetin well in fresh and dry Hangbaiju, and newer clustering frameworks for edible chrysanthemum were proposed specifically to avoid information loss from using too few indicators (Xu et al., 2022b; Jing et al., 2024). In practice, the best strategy is to screen harvest-drying combinations against a weighted panel that includes marker compounds, antioxidant activity, aroma or sensory data, morphology, and where needed antibacterial or other functional endpoints.

8 Conclusion and Prospects

An appropriate harvest period is the basis for high-quality production of Hangbaiju because the flowering stage directly determines the accumulation of phenolic acids, flavonoids, volatile substances, antioxidant capacity, and even the sensory identity of the final product. Studies on Hangbaiju showed that harvest stage significantly affected functional constituents, with total flavonoids and chlorogenic acid highest at the Hualei stage,

luteolin-7-O-glucoside and 3,5-O-dicaffeoylquinic acid highest at the Taiju stage, and the overall balance of constituents, yield, and harvest timing favoring Taiju as the best picking stage. Comparative work on Taiju and Duoju further showed that products harvested at different times differ substantially in phenolic profiles, antioxidant activity, flavor-related volatiles, and flavonoid composition, and metabolomic analysis identified 11 marker metabolites that can distinguish the two harvest periods. Related evidence from Fubaiju also showed that inflorescence diameter was negatively correlated with chlorogenic acid and 3,5-O-dicaffeoylquinic acid, and that smaller-diameter samples had higher polyphenol content and better sensory and antibacterial performance, which supports using maturity-linked morphological traits as operational harvest indices. More broadly, metabolomics-based work in *Chrysanthemum indicum* found that most varied active constituents were highest or relatively high at initial or full-bloom stages, reinforcing the general principle that intermediate maturity often provides the best quality foundation for chrysanthemum materials.

Rational drying methods are the key to maintaining Hangbaiju quality because drying not only removes moisture but also reshapes the retention of active compounds, aroma, color, antioxidant capacity, and microstructure. Direct evidence showed that drying process significantly affected the bioactive chemical contents and antioxidant activities of Xiaobaiju and Taiju, with suitable oven drying suggested at 60 °C for Xiaobaiju and 70 °C for Taiju, indicating that even closely related harvest materials require different drying settings. Across chrysanthemum studies, moderate or optimized drying usually outperformed uncontrolled heating: oven drying around 55–65 °C better balanced active and nutritional ingredients, pulsed vacuum drying preserved total phenolics, flavonoids, and marker compounds while improving antioxidant activity, and lower temperature or pulsed conditions better protected color and volatile components. Microwave-assisted and infrared-assisted methods also showed clear advantages, as microwave drying retained higher flavone, vitamin C, and soluble sugar under efficient conditions, microwave-hot air drying best preserved biologically active compounds and acetylcholinesterase inhibitory activity, and infrared-assisted or tempering-incorporated drying increased chlorogenic acid, luteolin, total phenolics, total flavonoids, antioxidant capacity, and volatile compounds while reducing energy use. At the same time, shade drying tended to preserve more flavonoids, phenolic acids, and terpenoids than heat drying, and even general chrysanthemum flower studies found better color, shape, and petal integrity under shade drying, which shows that the best method depends on whether priority is given to processing efficiency, medicinal components, or external appearance.

Standardization of harvest and processing is an important direction for upgrading the Hangbaiju industry because current quality differences across batches cannot be fully controlled by origin labels or traditional experience alone. Quality evaluation studies showed substantial variation even among commercial *Chrysanthemum morifolium* samples, with fingerprint similarities ranging from 0.640 to 0.956, and demonstrated that relying mainly on habitat and processing history while neglecting bioactive ingredients can lead to deviation in quality assessment. More robust strategies already exist: chromatographic fingerprinting combined with multi-component quantification, antioxidant evaluation, and spectrum-effect analysis identified Q-markers such as chlorogenic acid, 3,5-O-dicaffeoylquinic acid, 4,5-O-dicaffeoylquinic acid, and kaempferol-3-O-rutinoside, while integrated chromatogram-effect approaches and antioxidant activity-fingerprint models provided practical frameworks for quality assessment and authentication. Rapid detection technologies also support industrial upgrading, since near-infrared hyperspectral imaging predicted luteolin and quercetin in fresh and dry Hangbaiju, and fluorescence spectroscopy with chemometrics achieved high-accuracy origin authentication and functional compound prediction in chrysanthemum materials. Future optimization should therefore establish standardized harvest criteria centered on maturity stage, build process-specific drying protocols for different product goals, and adopt weighted multi-index models such as AHP, clustering, and chemometric authentication to achieve stable grading, premium branding, and higher-value utilization of Hangbaiju.

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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