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Physiological Study on Root Adaptation and Recovery of Tissue-Cultured *Anoectochilus roxburghii* Seedlings after Transplanting

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Abstract *Anoectochilus roxburghii*, a rare medicinal plant, has attracted widespread attention for its scarce resources and high cultivation difficulty. Tissue culture rapid propagation, has become the main seedling raising method, but the adaptation and recovery of the root system after transplantation remain a bottleneck. This study summarizes several regulatory mechanisms: the proportional relationship of hormones (IAA, CK, stellolactone), the accumulation of osmotic regulatory substances (proline, soluble sugar), and the activation of antioxidant systems (SOD, CAT, POD), which play an important role in alleviating transplant shock. Meanwhile, the upregulation of energy metabolism, the accumulation of secondary metabolites, and the absorption of mineral elements have been proven to be the key supports for the rejuvenation of root systems. Studies show that, a reasonable hormone ratio can shorten the recovery period of the root system. The accumulation of osmotic regulatory substances, and the enhancement of antioxidant enzyme activity, play a prominent role in the early recovery stage. In the later stage, light quality regulation and substrate improvement have shown obvious effects on the improvement of root function, and the overall adaptation of the plant. This study provides new ideas for the efficient cultivation and management of *A. roxburghii*, and also offers theoretical basis and guidance for its industrial promotion and resource protection.

Keywords *Anoectochilus roxburghii*; Transplant shock; Root recovery; Osmotic regulation; Growth hormone

1 Introduction

Anoectochilus roxburghii, often referred to as the "King Medicine" or "Golden Herb", is a highly valuable medicinal orchid. In traditional Chinese medicine, it has been widely applied, for its various pharmacological activities, including antioxidant, liver-protective, anti-diabetic and anti-inflammatory effects (Huang et al., 2022; Zhang et al., 2025a). It is rich in various bioactive components, like kinsenoside, flavonoids and polysaccharides, and is widely used in health foods, medicines and functional beverages (Chen et al., 2020; Zhang et al., 2020a; b; Huang et al., 2022).

Because of the endangered status of wild *A. roxburghii*, and the need for stable quality, tissue culture has become the main propagation method. This method can achieve rapid reproduction, maintain genetic consistency, and produce disease-free plants (Wang et al., 2022), and plays a role in meeting industrialization demands, and protecting germplasm resources.

But, when transplanting *A. roxburghii* tissue culture seedlings to a non-sterile environment, transplanting shock often occurs, containing root damage, water loss and impaired nutrient absorption, thereby significantly reducing the survival rate (Shao et al., 2014). The root systems of seedlings cultured in vitro, are fragile and are prone to be affected by environmental stress, at the time of the domestication process. Survival and growth after transplantation, are affected by various factors, like substrate composition, light intensity and quality, humidity, and the presence of beneficial or harmful microorganisms (Shao et al., 2014; Ye et al., 2020; Zhang et al., 2020a). If the conditions are not ideal, it will lead to poor adaptability of the root system, increased susceptibility to diseases, and a decrease in the accumulation of medicinal components.

This study attempts to reveal the physiological, and molecular response mechanisms of the root system of *A. roxburghii* to transplanting stress, with a focus on the activity of antioxidant enzymes, the accumulation of

osmotic regulatory substances, and the changes in gene expression, that promote root adaptation and recovery. By integrating the research results on substrate optimization, light regulation and the interaction of beneficial microorganisms, this study hopes to propose practical strategies to enhance the survival rate of transplanting, and promote the vigorous growth of plants, thereby supporting the sustainable cultivation and industrial application of *A. roxburghii*.

2 Root Characteristics and Transplantation Effects

2.1 Structural and functional traits of tissue-cultured roots

The root systems of *A. roxburghii* cultured in tissue culture, exhibit distinct morphological characteristics. Compared with wild or conventionally propagated plants, their root volume, length and quantity have all increased. Especially the tetraploid variant, has a thicker root system and larger stomata, which may enhance its water absorption and nutrient absorption capacity (Huang et al., 2022; Zhang et al., 2025b). Under optimized in vitro culture conditions, like the combination of specific plant growth regulators and phoqualities, the vigorous development of root systems can be further promoted. Under the ideal protocol, the rooting rate can exceed 90% (Wang et al., 2022; Zhang et al., 2025b).

The root system of *A. roxburghii* in tissue culture, also had high metabolic activity, manifested as increased contents of amino acids, minerals and various bioactive components (e.g., *A. roxburghii* glycosides, flavonoids) (Ye et al., 2020; Huang et al., 2022; Wang et al., 2022). These root systems are sensitive to environmental signals, and can support acclimation and growth through metabolic regulation. The presence of beneficial endophytic fungi can further promote root metabolism, and the accumulation of secondary metabolites (Ye et al., 2020).

2.2 Physiological effects of transplantation

Transplanting can expose tissue culture seedlings to sudden changes in substrate, humidity and microbial environment, often resulting in decreased water and nutrient absorption capacity (Wang et al., 2022; Zhang et al., 2025b). This is because its root system has not fully developed, or has been damaged during the transplanting process, making it difficult to adapt to non-sterile environments, causing transplanting shock and reducing the survival rate.

Transplanting stress can damage the integrity of root cell membranes, increase their sensitivity to oxidative damage, and weaken their physiological functions. For instance, salt and phosphate stress can lead to an increase in reactive oxygen species levels in roots, while treatments, like the application of strigolactone, can alleviate such damage and help maintain the stability of cell membranes (Zhang et al., 2025a; Zhong et al., 2025).

2.3 Necessity of root recovery

The powerful root regeneration ability, is an important feature for *A. roxburghii*, to overcome transplanting shock. Efficient root regeneration, can restore the functions of water and nutrient absorption, maintain metabolic activity, and promote the accumulation of medicinal components (Ye et al., 2020; Wang et al., 2022). So, optimizing the relevant schemes of rooting medium and environmental conditions, is very important for achieving maximum root regeneration (Wang et al., 2022).

The successful recovery of root system function, is closely related to the overall growth and survival of the plant. The enhancement of root vitality, can promote biomass accumulation, increase the content of secondary metabolites, and improve the survival rate after transplantation (Huang et al., 2022; Zhang et al., 2025b). At the same time, the utilization of beneficial microorganisms and customized light conditions, also can further promote root recovery and plant development (Wang et al., 2018; Ye et al., 2020).

3 Physiological Mechanisms of Root Adaptation and Recovery

3.1 Role of hormones in root recovery

Auxin, especially indole-3-acetic acid (IAA) and its analogues, such as indole-3-butyric acid (IBA) and naphthalene-acetic acid (NAA), are key hormones for the differentiation of roots and the formation of adventative roots in the tissue culture of *A. roxburghii*. Optimizing auxin concentration in rooting medium, can increase root

induction rate, root number and root elongation (Wang et al., 2022; Zhang et al., 2025b). Auxin promotes the formation of a strong root system, through stimulating the cell division and elongation of the root tip meristem, and enhances the plant's water and nutrient absorption capacity. Wang et al. (2022) found that, an appropriate hormone ratio (MS + 3 mg/L 6-BA + 0.5 mg/L NAA + 0.8 mg/L ZT + 0.2 mg/L 2,4-D), could significantly increase the induction rate (up to 89%) of prosphere structure (PLB) of *A. roxburghii.* And, the middle segment performs better than the base or top segment (Figure 1).

Cytokinin plays a role in maintaining root vitality, and promoting bud proliferation, when it is in balance with auxin. A reasonable ratio of cytokinin to auxin can simultaneously support the development of roots and buds, which is very important for the survival and growth of transplanted seedlings (Wang et al., 2022; Zhang et al., 2025b). Cytokinin helps maintain meristem activity and delay root senescence, ensuring that the root system remains physiologically active during the critical domestication stage. The interaction between auxin and cytokinin, is also involved in regulating the expression of genes related to cell cycle, differentiation and stress adaptation.

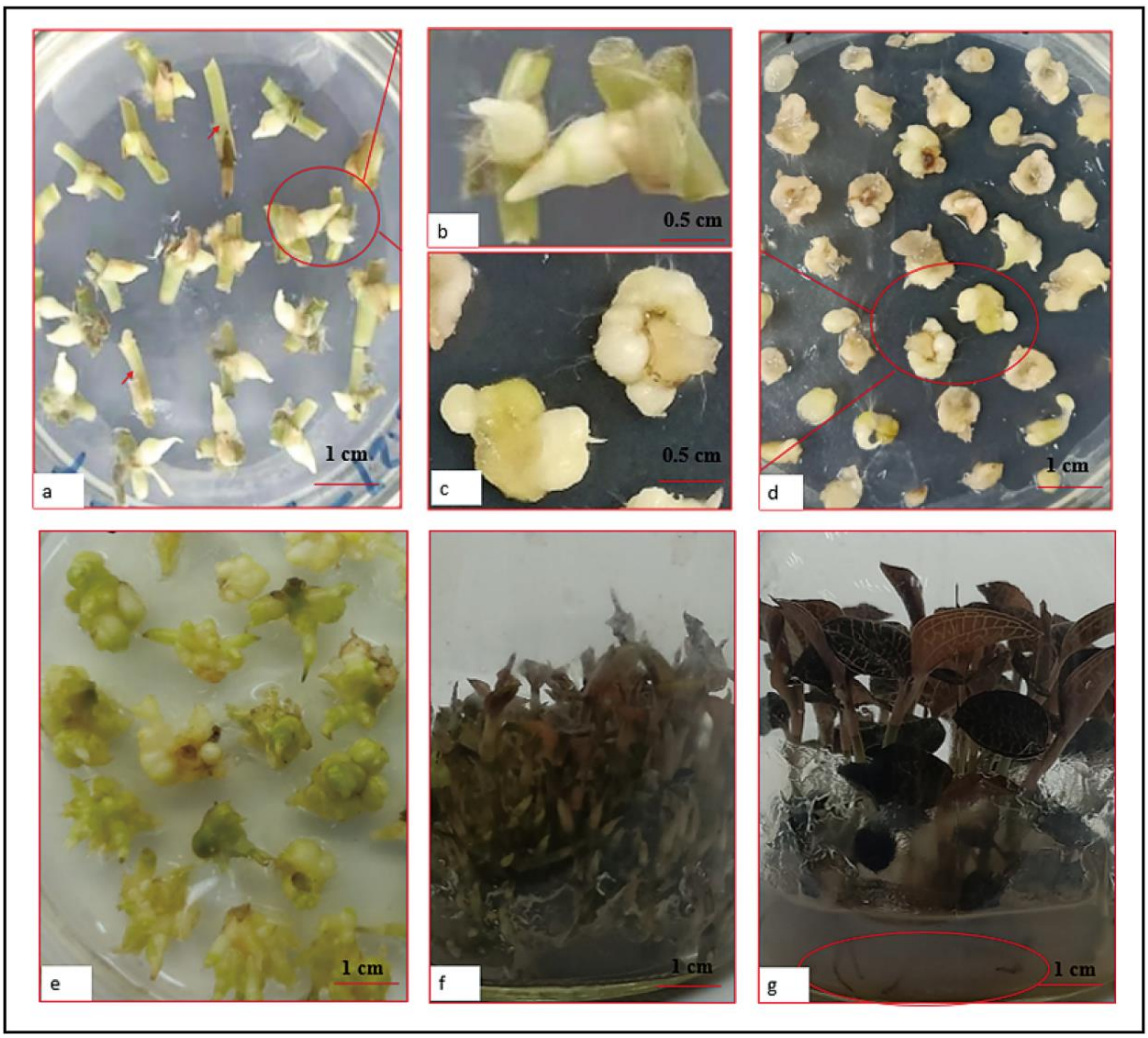


Figure 1 Induction, proliferation, and regeneration of *A. roxburghii* PLB. (a) Induction of *A. roxburghii* PLBs. The arrows indicate stem nodes near apical shoot. (b) Magnification (4×) of the selected area in panel a. (c) Magnification (4×) of the selected area in panel d. (d) Secondary PLB induction. (e) Mastoid PLB mass. (f) Shoot formation. (g) Root formation (the roots are within the circled region) (Adopted from Wang et al., 2022)

Image caption: The image clearly shows that under different tissue positions and culture conditions, the PLBs of *Anoectochilus roxburghii* can achieve efficient regeneration. Moreover, with appropriate hormone combinations (6-BA, NAA, ZT, 2,4-D) and light regulation, the transition from protocorm-like bodies to complete plantlets can be successfully accomplished, indicating the feasibility of this system for large-scale propagation and rapid seedling production (Adapted from Wang et al., 2022)

3.2 Osmotic regulation and water balance

When transplanting and under abiotic stress, like high temperature, drought or salt stress, the roots of *A. roxburghii* accumulate osmotic protective substances, such as soluble sugar and proline (Feng et al., 2023; Sun et al., 2023; Zhang et al., 2024). These compounds, help maintain cellular osmotic balance, stabilize proteins and membrane structures, and protect cells from dehydration and oxidative damage. Proline is particularly important. As a compatible solute, it can eliminate reactive oxygen species (ROS), and support the normal function of enzymes under stress conditions (Feng et al., 2023; Zhang et al., 2024).

Osmotic regulation through the accumulation of soluble sugar, and proline enables root cells to retain water, maintain turgor pressure, and maintain metabolic activity under environmental stress (Feng et al., 2023; Sun et al., 2023). This process is conducive to root elongation, cell division and the recovery of root function. Enhanced osmotic regulation directly contributes to improving the survival rate of transplanting and the growth of subsequent plants, as it can alleviate the impact of water deficiency and accelerate the acclimation process (Zhang et al., 2024).

3.3 Reactive oxygen species (ROS) balance and antioxidant systems

Transplanting and environmental stress, often lead to the excessive production of reactive oxygen species in roots, thereby causing oxidative damage to cellular components. *A. roxburghii* responds by up-regulating antioxidant enzymes, like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Sun et al., 2023; Zhang et al., 2024; Zhong et al., 2025). These enzymes work in synergy to eliminate ROS, reduce lipid peroxidation and protect root cells from oxidative damage. For instance, the application of strigolactone under phosphorus stress conditions, can promote root elongation and reduce ROS accumulation by enhancing antioxidant enzyme activity (Zhong et al., 2025).

Non-enzymatic antioxidants, such as polyamines and secondary metabolites (such as flavonoids), also play important roles in ROS detoxification, and cell protection (Sun et al., 2023; Ding et al., 2024). In *A. roxburghii*, overexpression of polyamine oxidase (PAOs), can enhance water stress resistance by regulating polyamine levels and strengthening antioxidant defense (Ding et al., 2024). Exogenous application of spermidine, has been proven to increase the content of endogenous polyamines, and the activity of antioxidant enzymes, further promoting root adaptation and recovery under water deficiency conditions (Sun et al., 2023).

4 Metabolic and Functional Changes in *Anoectochilus roxburghii* Roots

4.1 Energy metabolism regulation

After transplanting, the metabolic demands of the root system of *A. roxburghii* increase significantly, to support adaptation and regeneration. Transcriptomic analysis indicated that, genes related to energy metabolism (glycolysis, tricarboxylic acid cycle (TCA), and mitochondrial electron transport chain related genes, etc.), were all upregulated, suggesting enhanced mitochondrial respiration during root recovery and active growth stages (Zhang et al., 2020a; Yu et al., 2025).

The upregulation of energy metabolism pathways, leads to an increase in ATP production, which is important for root cell maintenance, ion transport and activation of stress response mechanisms (Zhang et al., 2020a; Chen et al., 2021). An adequate supply of ATP helps support the active transport process, cell membrane repair, and the synthesis of secondary metabolites. These processes collectively determine the successful adaptation, and survival of the root system after transplantation.

4.2 Secondary metabolite accumulation

Transplanting and environmental factors, like the interaction between light quality and microorganisms, affect the accumulation of secondary metabolites in the root system of *A. roxburghii*. Studies have shown that, arbuscular mycorrhizal fungi (*Ceratobasidium* sp.AR2) and endophytic fungi, can increase the contents of flavonol glycosides, flavonols, flavonoids and total phenols, by up-regulating key biosynthetic genes (Ye et al., 2020; Zhang et al., 2020a; b; Li et al., 2024).

Supplementation of blue light and red light, can also enhance the accumulation of total flavonoids and polyphenols, thereby improving the medicinal quality of the root system (Ye et al., 2017; Wang et al., 2018; Gam et al., 2020). Metabolomics and transcriptomics studies have found that, there are differences in the flavonoid and diterpenoid metabolite profiles among different organs, and roots show different secondary metabolite accumulation patterns from stems and leaves (Chen et al., 2020; Wei et al., 2022).

Flavonoids, phenols, diterpenoids and polysaccharides are not only antioxidant substances, but act as signal molecules, which can enhance the tolerance of root systems, to oxidative stress and abiotic stress (Wei et al., 2022; Yang et al., 2023; Jiang et al., 2025). The accumulation of these compounds, helps alleviate the damage caused by reactive oxygen species (ROS), maintain membrane stability, and enhance the overall stress resistance of plants during the domestication process. For instance, exogenous application of certain flavonoids, such as quercetin, has been proven to enhance antioxidant capacity and physiological indicators (Cui et al., 2023).

4.3 Mineral nutrient uptake and utilization

The root systems of *A. roxburghii* after transplantation, often face nutrient limitations, especially phosphorus deficiency. Studies have shown that, the application of strigolactone can alleviate hypophosphatemia stress by promoting root elongation, reducing oxidative damage, and possibly enhancing phosphorus absorption and utilization (Zhong et al., 2025). Beneficial rhizoidal bacteria and mycorrhizal fungi, can also improve nutrient assimilation efficiency, promote biomass increase and increase the content of active components (Wei et al., 2020; Ye et al., 2020).

Calcium and magnesium can help maintain the stability, and function of root cell membranes. Transcriptome data indicate that, calcium ion binding and related pathways are upregulated in response to environmental stimuli, which helps maintain cell wall integrity and participates in signal regulation during root adaptation (Li et al., 2024). These mineral elements, provide structural and functional resilience to root cells, promoting their successful domestication and continuous growth.

5 Phenotypic and Physiological Indicators of Root Recovery

5.1 Root morphological indicators

The morphological characteristics of the root system, are the main intuitive indicators for evaluating the root recovery of tissue-cultured *A. roxburghii* seedlings after transplantation. Studies have shown that, the optimized rooting protocol can achieve a higher number of single bud roots (like 2.62 roots per bud), and a higher rooting rate (92%), and increase root length and root surface area under suitable culture medium and light conditions (Zhang et al., 2025b). The symbiotic relationship between mycorrhizae and endophytic fungi, can further increase the number of roots, root length and overall biomass, reflecting the enhanced adaptability and nutrient absorption capacity of plants (Ye et al., 2020; Zhang et al., 2020a).

Microscopic observation of the root tip meristem, can reveal cell activity and the health status of the root system. Healthy meristem, which exhibits active cell division and an orderly tissue structure, is conducive to continuous root elongation and regeneration after transplantation. Immunocytochemical and histological studies have confirmed that, the symbiotic relationship of beneficial microorganisms can promote meristem activity, and root development without causing tissue damage (Ye et al., 2020).

5.2 Root vigor and physiological parameters

The triphenyltetrazolium chloride (TTC) reduction assay, is the standard method for evaluating the metabolic activity and vitality of root systems. The high TTC reduction rate indicates vigorous root respiration and strong activity, which is closely related to the successful domestication and growth of *A. roxburghii* (Wang et al., 2018). Light quality and exogenous treatment, can regulate root activity. For instance, under specific supplementary light conditions, the TTC reduction rate is significantly increased (Wang et al., 2018).

The electrolyte leakage test, can be used to measure membrane integrity. A low leakage rate indicates that, the root cell state is relatively healthy. Treatment methods that can alleviate oxidative stress (application of strigolactone or

exogenous polyamines), help maintain membrane stability and reduce the accumulation of malondialdehyde (MDA). MDA is an important marker of lipid peroxidation and cell damage (Sun et al., 2023; Zhong et al., 2025). So, these physiological parameters can serve as reliable indicators of root health and stress resistance.

5.3 Stress tolerance indicators

Antioxidant enzyme activities - including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) - are key physiological indicators, reflecting the stress resistance of root systems. Under conditions, like high temperature, salt stress or water shortage, the enhanced activities of SOD and CAT indicate that the ability of the root system to eliminate reactive oxygen species (ROS) is improved, enhancing the stress resistance of the roots (Zhang et al., 2024; Jiang et al., 2025). Exogenous treatments, such as the application of spermidine or rare earth elements, can further enhance the activities of these enzymes and promote root adaptation (Xu et al., 2016; Sun et al., 2023).

The increase in proline and soluble sugar content, is a reliable indicator for measuring the osmotic regulation, and stress adaptability of the root system of *A. roxburghii*. These compounds accumulate under environmental stress and can maintain cell turgor pressure, protect cell structure, and promote overall stress resistance and recovery (Zhang et al., 2020a; Sun et al., 2023; Zhang et al., 2024). In addition, the interaction between light quality and microorganisms, also can affect the accumulation of these osmotic protective substances (Wang et al., 2018; Ye et al., 2020).

6 Case Studies

6.1 Application of growth regulators in *Anoectochilus roxburghii*

The use of growth regulators, like indole-3-butyric acid (IBA) and 6-benzylaminopine (6-BA), can enhance the root regeneration of *A. roxburghii*. Zhang et al. (2025b) compared different hormone combinations, medium types and light quality conditions, to establish an efficient bud proliferation and rooting system. The experimental results show that, 1 mg/L BA + 0.05 mg/L NAA is the best hormone treatment combination, increasing the number of buds and the induction rate. In terms of medium selection, the B5 formula is more effective than MS and its diluted formula. In the photolysis treatment, the proliferation efficiency, was the highest under red light conditions, and both the number of buds and the induction rate reached the optimal level. During the rooting stage, the combination of MS medium and 1 mg/L IBA + 1 mg/L NAA performed the best, with significant improvements in the number of roots, survival rate and growth height. Eventually, the survival rate of domestication and transplantation reached 80% (Figure 2).

Exogenous application of plant hormones and rare earth elements, like $La(NO_3)_3$, $Ce(NO_3)_3$), can enhance the activities of antioxidant enzymes (SOD, CAT, POD), reduce the content of malondialdehyde (MDA), and improve root vitality and stress resistance during regeneration (Xu et al., 2016). Besides, treatment with strigolactone can alleviate low phosphorus stress, and improve plant adaptability by reducing oxidative damage and promoting root elongation (Zhong et al., 2025).

6.2 Effects of substrates on *Anoectochilus roxburghii* root recovery

The composition and aeration of the substrate, have a significant impact on the root regeneration of *A. roxburghii*. Studies have shown that, the substrate ratio of peat soil: river sand: peanut shell = 4:2:2, can achieve the highest survival rate, the longest root length and the largest root diameter, indicating that a well-aerated substrate, is conducive to promoting the vigorous development and regeneration of the root system (Zhu et al., 2019). Hydroponic systems with cotton layers or coconut coir substrates, can also promote the growth of stems, leaves and roots, and increase biomass and the content of lycoposide, further demonstrating the importance of substrate structure and aeration for optimal root recovery (Luan et al., 2025).

Nutrient-rich substrates can increase the survival rate, and enhance the vitality of the root system as well, which is manifested as an increase in plant height, fresh weight and root count (Zhu et al., 2019; Luan et al., 2025). Substrate selection, also affects the accumulation of secondary metabolites, like flavonoids and polyphenols, which are closely related to plant health and stress resistance.

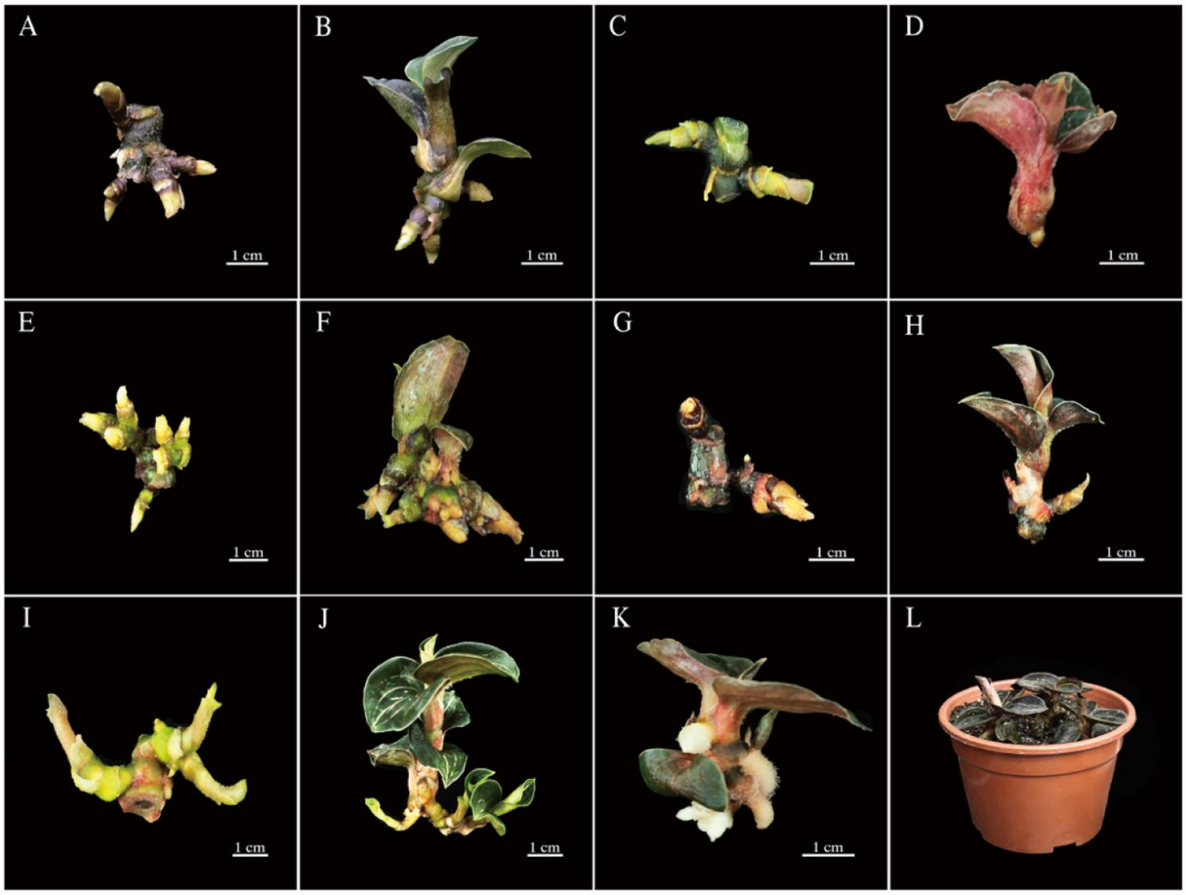


Figure 2 All figures from the micropropagation process. A. Shoot proliferation from stem segments, which were cultured on MS medium supplemented with 1.0 mg/L BA + 0.05 mg/L NAA under cool white light for 60 d. B. Shoot proliferation from terminal buds, which were cultured on MS medium supplemented with 1.0 mg/L BA + 0.05 mg/L NAA under cool white light for 60 d. C. Shoot proliferation from stem segments, which were cultured on 1/4 MS medium for 60 d. D. Shoot proliferation from terminal buds, which were cultured on 1/4 MS medium for 60 d. E. Shoot proliferation from stem segments, which were cultured on B5 medium for 60 d. F. Shoot proliferation from terminal buds, which were cultured on B5 medium for 60 d. G. Shoot proliferation from stem segments, which were cultured under R:B = 1:1 for 60 d. H. Shoot proliferation from terminal buds, which were cultured under R:B = 1:1 for 60 d. I. Shoot proliferation from stem segments, which were cultured under R for 60 d. J. Shoot proliferation from terminal buds, which were cultured under R for 60 d. K. Rooting in MS medium supplemented with 1.0 mg/L IBA + 1.0 mg/L NAA under cool white light for 50 d. L. Well-rooted plants were transplanted into pots with 3:1 peat and vermiculite for 30 d (Adopted from Zhang et al., 2025b)

7 Mechanistic Insights into Root Adaptation

7.1 Integrated mechanisms of root recovery

The root adaptation of *A. roxburghii*, involves the interaction of complex physiological and biochemical processes. Under stress conditions, such as low phosphorus or high temperature, the root system will show changes in the activity of antioxidant enzymes, like SOD, CAT, POD, accumulation of osmotic protective substances (proline, soluble sugar), and regulation of reactive oxygen species (ROS) levels. These reactions are closely linked: enhanced antioxidant activity helps to reduce oxidative damage, while osmotic protective substances stabilize cell structure and maintain metabolic function (Zhang et al., 2024).

Plant hormones, especially strigolactone, play a role in coordinating root adaptation. It can promote root elongation, regulate phosphorus absorption, and reduce oxidative stress (Zhong et al., 2025). Transcriptomic analysis revealed that, hormone signaling pathways, antioxidant systems, and metabolic networks, including protein and secondary metabolite synthesis, were synergistically regulated during stress adaptation, thereby ensuring root survival and recovery (Zhang et al., 2024; Zhong et al., 2025).

7.2 Relationship between root recovery and whole-plant growth

Root recovery, directly affects the growth of the aboveground part, through the root-stem signaling mechanism. For instance, the changes in root hormone levels and nutrient status, induced by stress will be transmitted to the above-ground parts, thereby affecting plant height, leaf development and flowering time. Transcription factors, like ArHDZ22, can simultaneously regulate the responses of roots and stems, integrating environmental signals with internal signals to optimize the overall adaptability of the plant (Zhang et al., 2024; 2025a).

A healthy root system can enhance the absorption of water and nutrients, supporting higher photosynthetic efficiency and biomass accumulation. Under optimal conditions or targeted treatments, such as sololactone and LED light, the improvement of root activity, will lead to an increase in chlorophyll content, improved photosynthetic performance, and ultimately achieve higher yield and medicinal quality (Gam et al., 2020; Zhang et al., 2024; Zhong et al., 2025).

7.3 Implications for industrial cultivation of *A. roxburghii*

Successful industrial cultivation, depends on optimizing substrate composition, hormone treatment and environmental conditions to promote vigorous root recovery. Schemes that can enhance root vitality, antioxidant capacity and nutrient absorption, are important for cultivating high-quality transplanted seedlings with strong vitality and growth potential (Zhang et al., 2025b; Zhong et al., 2025).

Adopting environmentally friendly measures - such as using LED light sources, to optimize growth and the accumulation of secondary metabolites, as well as rationally applying targeted hormone treatment - can not only increase yield and quality, but reduce environmental impact. The strategies provide strong support for the sustainable large-scale production, and germplasm resource conservation of *Anoectochilus roxburghii* (Gam et al., 2020; Zhang et al., 2025b).

8 Concluding Remarks

After transplantation, the seedlings of *A. roxburghii* obtained through tissue culture, will undergo a series of root physiological changes, including the regulation of hormone signals, the adjustment of nutrient absorption patterns, and the modification of antioxidant responses. These changes are coordinated, by the interaction of hormones such as auxin, cytokinin, ethylene and abscisic acid, thereby affecting root elongation, meristem activity and stress adaptation.

The enhancement of antioxidant enzyme activity and the accumulation of osmotic protective substances, such as proline and soluble sugar, help alleviate oxidative stress and promote root regeneration. The signal exchange between the roots and stems further balances the distribution of nutrients and hormones, ensuring the overall recovery and growth of the plant. It also pointed out that key factors, containing auxin, cytokinin, strigolide, nitric oxide, and transcription factors, that regulate root structure and stress response, are integrated with metabolic and antioxidant pathways, playing a decisive role in the adaptation and recovery of the root system after transplantation.

But, this study remains more at the physiological and biochemical levels, and there is still a lack of in-depth exploration of the mechanisms at the molecular level. For instance, changes in gene expression or signal networks at the transcriptome and proteome levels, have not yet been systematically analyzed. This also limits the precise localization of specific genes and pathways. Furthermore, most of the results are derived from greenhouse, or laboratory conditions and may not fully reflect the complexity of the field environment. Soil microbial communities, nutrient fluctuations and climate differences in the wild environment may all lead to different adaptation outcomes.

Future research should integrate transcriptomics, proteomics and metabolomics, to reveal the molecular networks of the transplanting adaptation process, and optimize substrates, hormones and acclimation protocols under field conditions. Meanwhile, it is also necessary to explore eco-friendly and sustainable cultivation models, to enhance root vitality, increase survival rates and medicinal quality, thereby promoting the large-scale production and industrial application of *A. roxburghii*.

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Conflict of Interest Disclosure

The authors affirm 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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