Medicinal Plant Research 2024, Vol.14, No.6, 334-344
http://hortherbpublisher.com/index.php/mpr

Research Perspective Open Access

Role of Optimizing Transplantation Environmental Conditions in Improving the Survival Rate of Tissue-Cultured Seedlings of *Anoectochilus roxburghii* (Wall.) Lindl.

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Medicinal Plant Research, 2024, Vol.14, No.6 doi: 10.5376/mpr.2024.14.0028
Received: 15 Oct., 2024
Accepted: 23 Nov., 2024
Published: 08 Dec., 2024
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Preferred citation for this article:
Li C.Y., and Zhao X.Y., 2024, Role of optimizing transplantation environmental conditions in improving the survival rate of tissue-cultured seedlings of *Anoectochilus roxburghii* (Wall.) Lindl., Medicinal Plant Research, 14(6): 334-344 (doi: 10.5376/mpr.2024.14.0028)

Abstract *Anoectochilus roxburghii* (Wall.) Lindl. is highly valued in traditional medicine due to its various pharmacological activities. However, the low survival rate of tissue cultured seedlings after transplantation remains one of the main bottlenecks in achieving large-scale cultivation. This study focuses on the key environmental factors that affect the success of transplanting *A. roxburghii* flowers, such as light intensity, substrate composition, and mycorrhizal symbiosis. The results indicate that the blue red combination (BR) LED light source plays an important role in promoting seedling growth and flavonoid accumulation, which helps to enhance its medicinal value. Maintaining a suitable temperature and humidity environment can effectively alleviate stress during transplantation and enhance the adaptability of plants. The use of a specific ratio of substrate mixture can improve root development and substrate water retention performance, and increase the survival rate after transplantation. The study also pointed out that inoculation with specific mycorrhizal fungi (such as *Ceratobasidium* sp. AR2) can enhance the nutrient absorption and stress resistance of plants, further improving the colonization effect. This study provides a scientific basis for optimizing the transplanting conditions of *A. roxburghii*, which is helpful for its sustainable cultivation and resource protection, and provides useful references for the transplanting management of other medicinal plants.
Keywords *Anoectochilus roxburghii*; Tissue culture; Transplantation environment; Mycorrhizal symbiosis; Light regulation

1 Introduction

Anoectochilus roxburghii (Wall.) Lindl. is often called the "king of medicine" in China. It has long been used to treat liver disease, diabetes, and hypertension because it contains active ingredients such as hyperoside and flavonoids (Gam et al., 2020; Wang et al., 2022). Unfortunately, wild *A. roxburghii* has become scarce today, mainly due to the loss of its natural habitat and overharvesting (Zhang et al., 2015). Therefore, it is particularly important to protect this plant and cultivate it in a sustainable way.

To address this problem, scientists often use tissue culture technology. This method can quickly reproduce a large number of healthy and consistent plants (Lin et al., 2018). However, tissue culture also has some problems. One of the main problems is that when the plantlets are transferred from the laboratory to the outside environment, they will face many challenges. In the laboratory, the plants can get all the nutrients they need and are protected from pathogenic microorganisms. But once they enter the outside environment, they will face changes in temperature and humidity, as well as invasion by bacteria and fungi, all of which can cause the plants to dry out or even die (Shao et al., 2019).

These lab-grown seedlings often have weak roots. They struggle to deal with outdoor conditions (Krasowski, 2003; Qin et al., 2022). Also, normal transplanting methods don't give them the special care they need. That makes it harder for them to survive. Because of this, it's tough to grow *A. roxburghii* on a large scale for medicine or conservation.

Researchers have been actively exploring more effective methods to improve the survival rate of tissue cultured seedlings of *A. roxburghii* after transplantation. They investigated the effects of environmental factors such as soil

type, lighting conditions, and air humidity on seedling survival from multiple perspectives (Zhu, 2015; Chen et al., 2021). It has found that using a combination of red and blue LED light sources can promote plant growth, and increase the content of beneficial active ingredients in their bodies (Gam et al., 2020). And the selection of soil type is also crucial. If the soil properties used are similar to those in its natural habitat, it can better promote root development and enhance the water retention capacity of the substrate (Wang et al., 2022; Al Alwani et al., 2023).

In the transplanting process, maintaining appropriate air humidity and taking reasonable shading measures can also help reduce the stress of external environment on plants, enable them to adapt to the new environment more smoothly as well (Zhang et al., 2020). However, although these improvement measures have shown positive effects in some studies, there are still studies indicating that they have not significantly improved the survival rate of seedlings. Thereby, it is currently unclear which method is most effective, and further research and verification are needed on response strategies under different environmental conditions.

In this study, we want to figure that out. We will test different conditions during transplanting to see how they affect the growth and survival of *A. roxburghii* seedlings from tissue culture. Our goal is to find simple and useful ways to help more of them live. Even though we focus on *A. roxburghii*, our results may also help with transplanting other types of medicinal plants grown in a similar way.

2 Tissue Culture Propagation of *Anoectochilus roxburghii*

2.1 Tissue culture techniques for *A. roxburghii*

Since *A. roxburghii* is a precious medicinal plant and its wild resources are becoming increasingly scarce, more and more people are beginning to propagate it artificially through tissue culture. This technology can not only quickly and safely reproduce plants in large quantities, which is beneficial to the protection of the species, but also promotes its industrial development. At present, most tissue culture experiments use Murashige and Skoog (MS) medium, a special medium containing a variety of nutrients. In order to improve its effect, researchers usually add plant growth regulators to the culture medium. Some studies have found that adding rare earth elements such as $La(NO_3)_3$ and $Ce(NO_3)_3$ can promote the growth of buds and make seedlings stronger. But the amount of addition is crucial - the right amount is beneficial, but excessive amounts can harm the plants (Xu et al., 2016).

The basic steps of tissue culture are relatively simple. The seeds are sterilized and then cultured in a sterile experimental environment. When the seeds germinate, healthy seedlings are selected and replicated in large numbers through asexual reproduction to obtain enough plants for large-scale cultivation (Chen and Liu, 2015). In order to further improve the development of buds and roots, researchers are also constantly optimizing culture conditions. Some studies have shown that using low-concentration MS culture medium and rationally regulating the content of plant hormones such as cytokinins and auxins can help promote the growth of buds and roots (Zhang et al., 2015). However, due to different experimental conditions, research results often vary, so a method that works in one laboratory may not work well in another laboratory.

2.2 Common issues affecting tissue-cultured seedlings' survival rate

Although there have been some advances in tissue culture techniques for *A. roxburghii*, successfully transplanting seedlings from the laboratory into soil still faces many challenges. The main problem occurs when the seedlings are exposed to drastic changes in outdoor humidity and temperature after leaving the stable laboratory environment. If these environmental differences cannot be properly addressed, many seedlings may wither and die (Chen et al., 2017).

Zou (2015) pointed out that selecting appropriate soil ratios, such as mixing fine sand with peat, can promote better root growth. This substrate is closer to the natural growth environment of *A. roxburghii*, which helps to improve the survival rate after transplantation. In addition to environmental stress, diseases are also a key issue. Seedlings cultured in tissue culture are particularly sensitive to external pathogens and are particularly susceptible to bacterial infections from unclean tools or materials (Zhang et al., 2021). Therefore, in the early stages of tissue culture and domestication, it is necessary to strictly ensure aseptic operation and finely control the culture environment.

It is interesting that some fungi living inside plants, known as endophytic fungi, may have the potential to help improve seedling survival rates. Studies have shown that these fungi can not only reduce the incidence of diseases, but also promote seedling growth. Some fungal strains have been found to increase plant size and enhance the content of beneficial compounds in their bodies (Ye et al., 2020). However, this positive effect cannot be stably exerted in all environments, and its effect is greatly influenced by external conditions and strain types. Therefore, it still needs to be carefully evaluated in practical applications.

2.3 Factors influencing growth during *in vitro* propagation

The growth of *A. roxburghii* is influenced by various factors at the time of tissue culture. One of the most critical factors is the formulation of the culture medium, in which the type and concentration ratio of growth hormone. Wang et al. (2022) found that a reasonable combination of plant hormones like 6-benzylaminopurine (BA), alpha naphthylacetic acid (NAA), and zeatin (ZT) is crucial for inducing bud formation and proliferation. If the hormone concentration is too high or too low, it may lead to slow growth or decreased plant quality. So, in practical operation, maintaining precise balance between hormones is the key to ensuring stable and efficient tissue culture results.

Light is also an important factor affecting the growth of tissue culture. Related studies have found that LED light sources with specific wavelengths, especially the combination of red and blue light, can not only promote rapid growth of seedlings, but also significantly enhance the synthesis of beneficial compounds such as flavonoids (Figure 1). This type of light stimulates the expression of related genes, thereby enhancing the medicinal value of *A. roxburghii* (Gam et al., 2020).



Figure 1 Effects of different light conditions on the *A. roxburghii* morphology; (a): fluorescent light; (b): red; (c): blue; (d): BR (one blue: four red); (e): BRW151 (one blue: five red: one white); (f): BRW142 (one blue: four red: two white). Scale bars = 1 cm (Adopted from Gam et al., 2020)

Image caption: The results in the figure indicate that compared to single-wavelength red or blue light, plants grown under BR light exhibit more robust growth, characterized by larger leaf areas, more upright stems, and a greater number of leaves. In contrast, plants treated with red light have thinner and weaker stems, while those under blue light have smaller leaves and restricted morphological development. This figure demonstrates that the combination of blue and red light can promote the growth and biomass accumulation of *A. roxburghii*, providing experimental evidence for optimizing artificial light cultivation (Adapted from Gam et al., 2020)

Some scientists have also tried growing the plants in special systems called continuous immersion bioreactors. These setups keep the plants in liquid and have been shown to increase both plant size and the amount of useful compounds they make (Jin et al., 2017). But, like with other methods, the results can be different depending on how the system is used. More work is still needed to figure out the best setup.

3 Environmental Factors Affecting Transplantation Success

3.1 Temperature and humidity control

Temperature and humidity play a big role in whether *A. roxburghii* seedlings survive after being moved out of the lab. In the beginning, the seedlings lose water easily and get stressed. So, keeping the air humid and steady helps

them adjust better (Susila et al., 2018). But too much moisture isn't always good. If the air stays too wet for too long, it can cause mold or fungal infections. That's why it's important to keep humidity at the right level—not too high, not too low.

Studies have shown that when humidity stays stable, the seedlings have a better chance of surviving. This kind of environment lowers stress and helps them settle in more smoothly during the early stages (Shao et al., 2014; Zhang et al., 2021).

Temperature is also important. Sudden changes can make the plants wilt or stop growing. Using greenhouses or climate-controlled rooms helps keep the temperature steady. These spaces protect the seedlings and help them get used to the outside world more gently (Zhang et al., 2021). Of course, problems can still happen—like equipment breaking or weather changes—but overall, controlled environments are one of the best ways to support healthy growth and improve survival.

3.2 Light intensity and photoperiod adaptation

The intensity of light and the duration of light exposure per day are important for the growth of golden thread vine seedlings. Reasonable setting of these two parameters can help improve the efficiency of photosynthesis and promote the healthy growth of plants. It has shown that the use of LED lights combine red light and blue light is particularly beneficial for golden thread vine seedlings. This composite lighting method can not only promote the synthesis of flavonoids, but also improve the growth of leaves and stems (Gam et al., 2020; Chen et al., 2021). In contrast, the effect of using red light or blue light alone is not ideal and cannot significantly improve the quality of plants.

The time that seedlings receive light every day, that is, the "photoperiod", also affects their growth performance. Studies have found that an appropriate photoperiod helps plants grow vigorously and increases the accumulation of useful ingredients such as flavonoids and polysaccharides (Wang et al., 2022; Cao et al., 2024). However, if the light duration is too long or too short, it is easy to disrupt the physiological rhythm of the plant and weaken its growth potential. In a word, during the transplanting stage of tissue culture seedlings, special attention should be paid to the fine control of light intensity and light duration to help the seedlings better adapt to the new environment and promote their healthy growth.

3.3 Soil and substrate composition for optimal growth

The soil type or substrate ratio selected during the transplanting process of *A. roxburghii* seedlings has a decisive impact on the survival rate and growth status of the plants. A suitable substrate can maintain the healthy state of roots, provide sufficient nutrition for plants, and maintain a suitable water environment.

Research has shown that a 2:1 ratio of peat to river sand is an ideal cultivation substrate for young *A. roxburghii* seedlings. This ratio helps to improve the survival rate of seedlings and promote their more robust growth (Zhu, 2015; Shao et al., 2014). However, this ratio is not fixed, and other studies have also pointed out that adjusting the ratio appropriately according to local climate conditions or the type of seedlings selected can still achieve good results, so it has a certain degree of flexibility in practical applications.

Adding natural materials such as humus or bark in moderation can also help further improve the matrix structure. These types of organic compounds not only enhance the nutrient absorption capacity of plants, promote root development, but also provide support during plant growth and continuously release beneficial substances (Lazcano Bello et al., 2021).

Excessive use of organic materials may also have negative effects, especially leading to excessive substrate water holding capacity, affecting the normal respiration of roots and endangering plant health. Therefore, when selecting and configuring substrates, it is necessary to balance nutrient supply and water retention capacity, while ensuring good breathability and overall balance.

4 Role of Mycorrhizal Associations in Seedling Adaptation

4.1 Symbiotic relationships between *A. roxburghii* and mycorrhizal fungi

A. roxburghii often depends on helpful fungi to grow better and adapt after transplanting. Some common fungi that work well with its roots include *Ceratobasidium* sp. AR2 and *Epulorhiza* sp. These fungi help the plant take in more nutrients and can also increase the amount of useful compounds like flavonoids (Li et al., 2012; Zhang et al., 2020).

These fungi don't just live near the roots—they actually grow into the root tissues and form something called endotrophic mycorrhiza. This means the fungi and plant directly trade nutrients with each other. This close relationship helps the plant grow faster and become stronger. But not all fungi are helpful. Some don't do much, and others can even be harmful. It depends on the type of fungus and the surrounding environment.

Besides helping with nutrients, these fungi can also protect the plant from stress, like disease or bad weather (Zhang et al., 2020). When the fungi live in the roots, they can turn on certain plant genes that help the seedling deal with stress (Li et al., 2012; Ye et al., 2020). However, these benefits don't happen automatically. Whether or not the fungi can grow well with the plant depends on things like the soil type and how stable the environment is. So even with the right fungi, good conditions are still needed for them to really help the seedlings.

4.2 Effects of mycorrhizae on nutrient uptake and stress resistance

Mycorrhizal fungi help *A. roxburghii* take in more nutrients by making the root system more effective. They expand the area that roots can use to absorb important elements like phosphorus and nitrogen (Diagne et al., 2020; Khaliq et al., 2022). This is especially helpful when the soil is poor in nutrients. Still, the results can differ depending on the type of fungus and the growing conditions. That's why it's important to choose the right fungi and apply them properly for each situation.

These fungi also help seedlings deal with stress from the environment, such as drought, salt, or extreme heat or cold. They do this in a few ways: they help the plant hold onto water, balance nutrients, and produce natural chemicals that protect against stress. These include antioxidants and hormones that support plant health.

Mycorrhizae also protect against disease. They take up space on the roots, making it harder for harmful microbes to attach. They also help turn on the plant's immune system. But this protection isn't perfect. If the environment becomes too harsh or the fungi don't fully connect with the roots, the plant can still get sick (Khaliq et al., 2022).

4.3 Application of mycorrhizal inoculation in transplantation success

Giving *A. roxburghii* seedlings a boost with helpful fungi before transplanting can really make a difference. When seedlings are treated with fungi like *Ceratobasidium* sp. AR2, they usually grow better. They tend to have more shoots, bigger root systems, and more overall weight (Zhang et al., 2020). This is especially helpful for tissue-cultured seedlings, which often have weak roots and struggle when moved outside the lab.

These fungal inoculations don't just help with growth. They also make the seedlings stronger and more able to handle the stress that comes with transplanting (Ye et al., 2020; Zhang et al., 2020). The fungi help the plant take in more nutrients and deal with sudden changes in the environment.

Still, this method doesn't always work the same way. The results depend on which fungus is used, how it's applied, and whether the plant and fungus are a good match. Conditions like soil type and weather also matter. Even so, adding helpful fungi to the transplant process is a smart way to improve survival rates and grow healthier *A. roxburghii* plants.

5 Physiological and Biochemical Adjustments During Acclimatization

5.1 Changes in photosynthetic capacity and chlorophyll content

As *A. roxburghii* seedlings get used to life outside the lab, their ability to do photosynthesis and their chlorophyll levels change. Light plays a big role in these changes. When seedlings are grown under a mix of red and blue LED lights, they often show better photosynthesis. That's because these light colors help the plants make more

pigments (Gam et al., 2021). But the amount of light matters, too. A strong mix of red and blue light can help plants grow faster. Still, if the light is too bright, it can actually stress the seedlings instead of helping them (Chen et al., 2021).

Chlorophyll—the green pigment that helps plants use light—also goes up under red and blue LED lighting. More chlorophyll means the plant can take in more light and turn it into energy (Gam et al., 2020). However, plants don't adjust right away. When the lighting changes suddenly, photosynthesis may drop at first before it gets better. That's why it's important to give the seedlings a balanced light setup. It helps them slowly get used to the new conditions and grow more successfully after transplanting.

5.2 Antioxidant enzyme activities and stress tolerance mechanisms

Antioxidant enzymes play an important role in alleviating environmental stress during the transplantation of *A. roxburghii* seedlings. These enzymes can effectively help plants resist cell damage caused by adversity, thereby enhancing overall stress resistance. At the same time, changes in the external environment can also affect the synthesis of beneficial compounds with antioxidant activity in plants, such as flavonols in polysaccharides and flavonoids, which have strong ability to scavenge free radicals and play a positive role in the plant's antioxidant defense system.

Jin et al. (2018) pointed out that adding yeast extract to the culture medium can enhance the antioxidant capacity of *A. roxburghii* seedlings, help them remove harmful substances such as free radicals, and reduce cell damage. However, the effectiveness of this effect is greatly affected by variety differences and specific cultivation conditions, and the application plan needs to be flexibly adjusted according to the actual situation during the application process.

Optimizing the cultivation system is also one of the important strategies to enhance plant stress resistance. For example, adding appropriate combinations of plant hormones to improved MS medium can significantly promote root and shoot development, helping seedlings to smoothly transition from the laboratory to the natural environment (Ru, 2015). Although various measures have been taken to enhance the stress resistance of *A. roxburghii*, sudden climate change or disease invasion during the transplanting process is still difficult to completely avoid. Therefore, continuous optimization of management methods and cultivation strategies remains an important guarantee for ensuring successful transplantation and healthy growth of seedlings.

5.3 Root system development and water uptake efficiency

Having a strong root system is key for *A. roxburghii* seedlings to take in water and survive after being moved out of the lab. One way to improve root growth is by adjusting the growth medium. Adding the right amount of plant hormones like naphthalene acetic acid (NAA) and 6-benzylaminopurine (6-BA) can help roots grow better (Ru, 2015). When the roots are strong, the plants absorb more water, which helps them grow and survive during acclimatization. However, these hormones must be used carefully. If the amounts are too high, they can actually slow down growth instead of helping.

The type of soil or substrate also makes a big difference. Studies show that mixes with peat and bark create a good environment for roots. This kind of substrate helps the plants grow stronger and survive better after transplanting (Zhu, 2015). Still, one mix doesn't work for every situation. You might need to change it slightly depending on the local climate or soil conditions.

6 Case Studies

6.1 Study on the effects of *Ceratobasidium* sp. AR2 on the growth of *A. roxburghii* tissue culture seedlings

Suitable environmental conditions for transplanting, including soil substrate, moisture, light, and utilization of symbiotic microorganisms, are essential for improving the survival of *A. roxburghii* tissue culture seedlings. A study verified the promotional effect of *Ceratobasidium* sp. AR2 on the growth and transplantation acclimatization of *A. roxburghii* tissue culture seedlings through a co-culture experiment (Zhang et al., 2020). In the treatment group inoculated with AR2, *A. roxburghii* plants exhibited significantly faster growth rate as

evidenced by increased root number, elevated biomass, and increased number of new shoots (Figure 2). A strong root system is essential for transplanting of tissue culture seedlings, as a good root structure improves water and nutrient uptake, thereby increasing the plant's ability to adapt to changes in the external environment. In addition, the changes in leaf color indicated that *Ceratobasidium* sp. AR2 induced the accumulation of flavonoids in *A. roxburghii*. These secondary metabolites may play an important role in improving antioxidant capacity and enhancing stress tolerance, which may help the plants to better adapt to environmental stresses after transplanting.

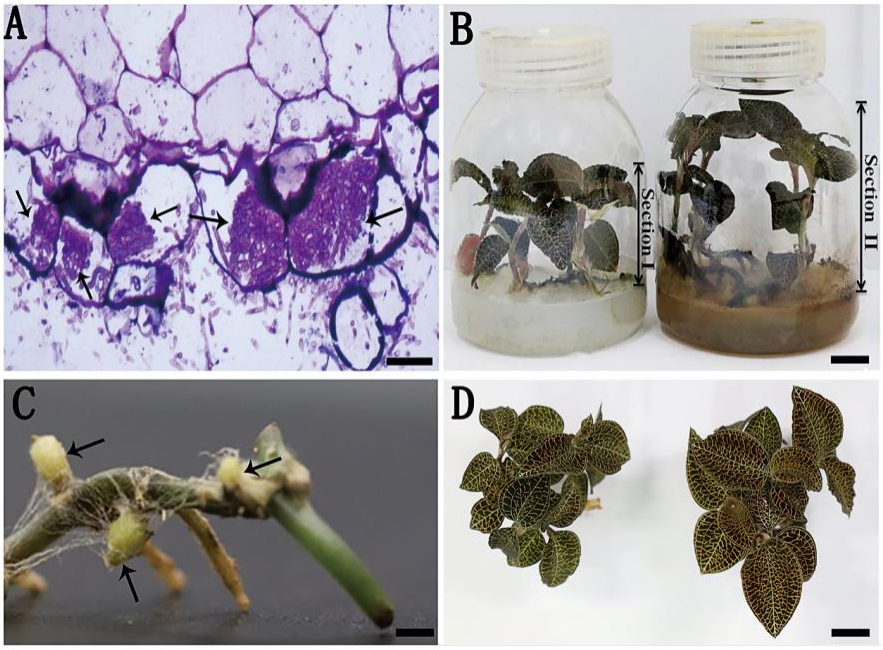


Figure 2 *A. roxburghii* inoculated with AR2 and uninoculation. (A) The semi-thin section of root of *A. roxburghii* after 1 week of symbiotic cultivation; arrows represent peloton, scale bar = 50 mm. (B) *A. roxburghii* grown in tissue culture bottle for 4 months inoculated with AR2 (Section II) and uninoculation (Section I). Section I represents the upper plantlets of the medium for the control; Section II represents the upper plantlets of the medium for the treatment; scale bar = 1 cm. (C) Local morphology from the plantlet of the treatment group; arrows represent new buds, scale bar = 1 cm. (D) Contrast of leave color between the control group (the left) and treatment group (the right) after 4 months of culture, scale bar = 1 cm (Adopted from Zhang et al., 2020)

This study provides a new strategy for the artificial cultivation of *A. roxburghii*, i.e., by adjusting the symbiotic microorganisms and transplanting environmental conditions, such as suitable soil substrate and water and fertiliser management, to improve the health status of the tissue culture seedlings and the survival rate of transplanting. In the future, the mutualistic mechanism between mycorrhizal fungi and *A. roxburghii* can be further explored and combined with environmental optimisation techniques to enhance the benefits of large-scale cultivation of *A. roxburghii*.

6.2 Commercial-scale transplantation and survival enhancement strategies

On a commercial scale, the transplant success rate of *Anoectochilus roxburghii* has been significantly improved through various strategies. For example, Wang et al. (2022) enhanced the tissue culture efficiency of *A. roxburghii* by optimizing the induction, proliferation, and regeneration (IPR-PLB) system of protocorm-like bodies (PLBs). The study found that using a culture medium containing MS + 3 mg/L 6-BA + 0.5 mg/L NAA + 0.8 mg/L ZT + 0.2 mg/L 2,4-D increased the PLB induction rate to 89%, with a secondary PLB induction rate of 120% and proliferation rates (in terms of both quantity and biomass) reaching 400% and 350%, respectively (Figure 3). The experiments demonstrated that PLBs proliferate optimally under dark or low-light conditions, while light exposure facilitates their differentiation into complete plantlets. This study established an efficient IPR-PLB system, improving the micropropagation efficiency of *A. roxburghii*, contributing to the resolution of wild resource shortages, and promoting its industrial-scale cultivation.

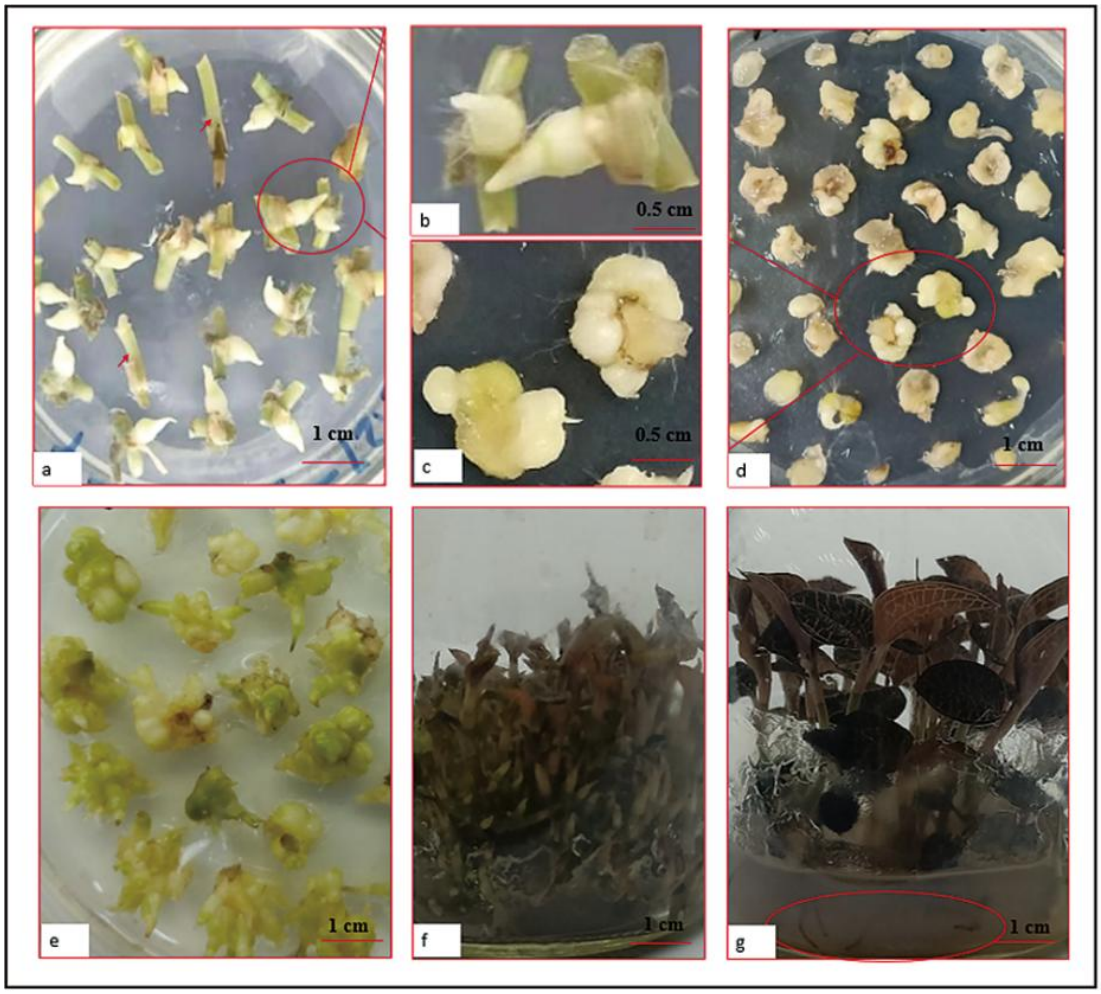


Figure 3 Induction, proliferation, and regeneration of *A. roxburghii* PLB. (a) Induction of *A. roxburghii* PLBs. The arrows indicate stem nodes near apical shoot. (b) Magnification (4×) of the selected area in panel a. (c) Magnification (4×) of the selected area in panel d. (d) Secondary PLB induction. (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 results showed that under optimized culture conditions (MS + 3 mg/L 6-BA + 0.5 mg/L NAA + 0.8 mg/L ZT + 0.2 mg/L 2,4-D), the PLB induction rate reached 89%, and the secondary PLB induction rate reached 120%. Dark or low light conditions facilitated the proliferation of PLBs, while light promoted their differentiation to intact plants.The high proliferation rate of PLBs provides an effective strategy for the rapid propagation of *A. roxburghii*, and also supports the potential of PLBs as a platform for secondary metabolite production (Adapted from Wang et al., 2022)

Another successful protocol focused on the large-scale in vitro propagation of *A. roxburghii*, which is crucial for both conservation and commercial purposes. This method utilized nodal segments from field-grown plants as explants and optimized the shoot generation and proliferation processes. The study identified half-strength Murashige and Skoog (MS) medium supplemented with 1.5 mg/L 6-benzyladenine (BA) as optimal for shoot induction, achieving a formation rate of 91.67% (Zhang et al., 2015). For rooting, a medium containing 0.6 mg/L NAA, 0.3 mg/L indole-3-butyric acid (IBA), and 100 mg/L banana mashes was used, resulting in a root induction rate of 93.33%. The rooted plantlets were then successfully acclimatized in a mixture of sterile sand and peat soil, achieving a survival rate of 90.2%.

7 Future Perspectives and Challenges

7.1 Potential improvements in transplantation protocols

Even though transplanting *A. roxburghii* seedlings has gotten better over time, there's still a lot of room to improve the process. One way to do this is by fine-tuning the soil mix. For example, Zhu (2015) found a mix of peat, bark, and other materials in a 14:1:5 ratio led to a high survival rate—up to 98.1% after 90 days. That's a

great result. But what works in one place might not work as well in another. So, testing different mixes or adding other helpful materials could lead to even better growth.

Besides the soil, getting the right temperature and humidity also matters a lot. Some researchers have used carefully planned experiments—like orthogonal designs—to find the best lighting setups for boosting growth and compound production (Chen et al., 2021). Using the same approach to fine-tune humidity or temperature might also help, but it's tricky to get consistent results in different environments. That's why more detailed studies are needed.

Another promising idea is using advanced micropropagation techniques. Some studies show that protocorm-like bodies (PLBs) can be used to grow more plants and even produce useful compounds like kinsenoside (Wang et al., 2022). However, it's not easy to add this method to regular transplanting steps just yet. It still needs more testing and adjustment.

7.2 Sustainable cultivation and conservation strategies

Since *A. roxburghii* is both valuable and endangered, finding better ways to grow and protect it is very important. One good method is to improve the growing conditions in the lab so the plant can make more useful compounds like polysaccharides and kinsenoside. Jin et al. (2018) showed that certain types of growing media, when combined with the right plant hormones, can help the plant produce more of these helpful substances in rhizome cultures. This means we can rely less on picking plants from the wild. But even with these advances, it's still hard to fully replace natural sources, because lab results can change from place to place.

Another key step is setting up strong and reliable ways to grow lots of plants. Using well-designed growth media and standard growing steps, we can produce large numbers of seedlings in lab conditions (Ru, 2015). This helps reduce the need to collect wild plants, which is good for both conservation and farming. Still, moving the lab-grown plants outdoors is not always easy. If the change isn't handled carefully, many seedlings might not survive.

7.3 Integrating AI and precision agriculture in transplantation optimization

Using artificial intelligence (AI) along with precision farming tools could help improve how *A. roxburghii* seedlings are transplanted. AI can look at a lot of growing data and find patterns that show the best conditions for healthy growth (Castillo, 2023; Kim and AlZubi, 2024). This helps farmers and researchers adjust their methods in real time to better match the plants' needs. Still, even the best AI predictions need to be tested in real-life situations, since plants don't always grow exactly as expected.

Precision agriculture uses tools like sensors and automated systems to control things such as temperature, humidity, and light. For example, light sensors can notice when the light is too weak or too strong and adjust it automatically. This helps reduce plant stress and supports better photosynthesis (Chen et al., 2021). But these systems aren't perfect—sometimes they break down or need to be recalibrated. Even with a few challenges, combining AI with smart farming tools offers a powerful way to improve transplant success. It can make growing *A. roxburghii* more efficient and also support its long-term protection.

Acknowledgments

The authors thank the two anonymous reviewers for their feedback on the manuscript of this study.

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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