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Case Study Open Access

Effects of Different Transplanting Substrates on the Survival Rate and Growth Quality of *Anoectochilus roxburghii* Tissue Culture Seedlings

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Abstract *Anoectochilus roxburghii* is a precious and rare medicinal plant with extensive uses in traditional Chinese medicine due to the richness of bioactive compounds and unusual pharmacological activities. Tissue culture seedlings are a primary tool for *A. roxburghii* cultivation, but their survival ratio and growth quality during the transplanting phase are determined by a series of factors among which the transplanting substrate selection is particularly crucial. With the technology of cultivating research being a key component of the discipline, the effects of different transplant substrates on plant growth have been of special interest. In this review, the biological properties of *A. roxburghii* and the progress in its bioactive compounds research are detailed systemically. It also explains the main mechanisms in which transplanting substrates impact plant growth, especially the interactions among substrate physical and chemical properties (e.g., water holding capacity, aeration, and nutrient supply) and seedling survival rate and growth quality. Moreover, it indicates successful experience and technical issues in substrate choice both at home and abroad and elaborates on how to optimize and manage substrates for promoting the industrial development of *A. roxburghii*. This review provides scientific citations for study and use of transplanting media of *A. roxburghii*, driving the development of successful cultivation technologies.
Keywords *Anoectochilus roxburghii*; Transplanting substrate; Tissue culture seedlings; Survival rate; Growth quality

1 Introduction

Anoectochilus roxburghii, or the "king of medicinal orchids," is one of the most precious medicinal plants with high bioactive ingredients, including flavonoids, polysaccharides, and glycosides. They display several pharmacological actions such as antioxidation, anti-inflammation, and immune regulation, hence making the plant a significant source of traditional Chinese medicine (Zhang et al., 2017). Due to its medicinal importance and increasing demand, its cultivation and research have been accelerated in the recent past. Natural populations of *A. roxburghii* have, however, greatly diminished in number due to excessive exploitation and destruction of habitat, and artificial cultivation is now essential for its conservation and rational use (Chen et al., 2019). Methods of tissue culture have proved to be efficacious for the multiplication of *A. roxburghii*, with a steady source of producing seedlings in large numbers and relieving pressure on natural resources (Wang et al., 2020).

The transplanting stage is one of the most crucial phases in *A. roxburghii* tissue culture seedling growth because the seedlings are sensitive to environmental factors and require special attention to develop and live. One of the most important challenges in this phase is selecting the right transplanting medium that will provide physical support, water-holding capacity, aeration, and nutrients to facilitate root development and general plant growth (Lin et al., 2021). Substrate conditions leading to excessive mortality, stunted growth, and seedling quality are the main constraints for commercial cultivation of the species.

The significance of transplanting the substrate is that it affects the surrounding chemical and physical environment of the seedlings, thus the health of the plant, nutrient absorption, and secondary metabolites production (Zhou et al., 2022). Despite advancements in tissue culture propagation techniques, there is no standardized optimized substrate for particular *A. roxburghii* requirements. This deficit should be bridged for enhancing the survival rate, quality of growth, and production of bioactive products in tissue culture seedlings that are essential to meet the demands of pharmaceutical use and large-scale commercial cultivation.

With reference to the research on the interaction between matrix properties and plant physiological response, the present work will identify key matrix properties that promote seedling performance (Chen et al., 2019; Lin et al., 2021). In addition, the research will develop scientifically based rules to propose appropriate transplanting substrate choice and optimization plans for research and industrial manufacture. The conclusions of this research will provide an excellent reference for clematis culture technology, promote the utilization of high-efficiency transplanting technology, and drive this medicine crop toward sustainable industrialization (Zhang et al., 2017; Zhou et al., 2022). By raising key questions during Melematis' early developmental stage, this work also has broader applications for tissue culture-based medicinal plant resource preservation and reproductive technology innovation.

2 Research Status of *Anoectochilus roxburghii* Tissue Culture Seedlings and Transplanting Substrates

2.1 Major bioactive compounds and medicinal value of *Anoectochilus roxburghii*

Anoectochilus roxburghii, a perennial herb of the family Orchidaceae, is famous due to its enormous medicinal virtues. The herb comprises a number of bioactive molecules responsible for its curative effects, such as anti-inflammatory, antioxidant, and immunomodulatory properties. These effects render it an exemplary plant in traditional medicine and contemporary pharmacology (Yao et al., 2024).

2.2 Physical and chemical properties of substrates and their effects on plant growth

Substrate should be selected to ensure proper growth and development of *Anoectochilus roxburghii* tissue culture seedlings. Some of the commonly applied substrates are fine sand, peat soil, and organic substrates such as peanut shells and bark. They provide nutrients, enhance water holding capacity, and provide improved air permeability, closely mimicking the plant growing conditions in the natural habitat. These conditions allow for root establishment and general health of the plant, thereby increasing the percentage survival of the transplanted seedlings (Ye et al., 2017).

2.3 Progress in domestic and international research on substrate selection for *Anoectochilus roxburghii*

Anoectochilus roxburghii substrate selection studies have been going on successfully, with researchers experimenting with various combinations to find the optimal conditions for growth and development. For instance, it has been discovered that a combination of decomposing soil and vermiculite is effective since it results in a high rate of survival when grown under shade conditions. In addition, the use of banana in culture media was discovered to enhance seedling growth, including leaf numbers, root growth, and fresh weight (Gam et al., 2020). All these findings hint at ongoing growth of substrate composition optimization for improved cultivation outcomes.

2.4 Technical challenges and development directions in transplanting substrate research

Even though a lot has been done, there are also some technical challenges in the research on substrate transplanting of *Anoectochilus roxburghii*. One of them is the maintenance of the quality and homogeneity of the substrates, which can shift depending on raw material sources and preparation methods. Extensive research is also needed to ascertain the long-term effects of substrates on yield and plant health. Future research needs to work towards developing standard substrate formulations and explore new materials to be utilized for further enhancing tissue culture seedlings growth and survival (Li et al., 2017).

3 Effects of Transplanting Substrates on the Survival Rate of Tissue Culture Seedlings

3.1 Mechanisms of substrate physical and chemical properties affecting survival rate

Physical and chemical properties of transplanting media are crucial in determining seedling survival rate of *Anoectochilus roxburghii* tissue culture seedlings. Peat, river sand, and bark have proved to contain ideal conditions for seedling survival since they hold water and provide good aeration. The combination of river sand and peat, for instance, offers a balanced substrate in which both water retention and drainage are encouraged, which is important in avoiding root rot and guaranteeing healthy growth. The chemical composition of the

substrates, for instance, pH and nutrient content, also plays a key role in seedling growth. A substrate pH of approximately 4.75 and suitable nutrient level is in favor of beneficial growth for *A. roxburghii* (Zhang et al., 2015).

3.2 Effects of water retention and aeration of substrates on survival

Aeration and water storage are essential factors to be considered for the survival of *A. roxburghii* seedlings. Mediums that find equilibrium between water storage and aeration, i.e., mixtures of peat and vermiculite, have been documented to lead to maximum survival by preventing waterlogging and providing an efficient oxygenation of roots (Xu et al., 2016). Use of substrates like bark and peat, with a capacity to provide aeration in addition to controlling water-holding ability, has been associated with survivability levels of 98.1% and higher in certain research reports (Wang et al., 2022). It is this equilibrium that has resulted in environments which will induce root disease to be shunned and instead promote roots to grow favorably.

3.3 Correlation analysis between substrate types and survival rates

Different substrates have been reported to be linked with varying survival rates of *A. roxburghii* seedlings. Experiments revealed that substrates that contain peat, river sand, and peanut shell in some ratio have been reported to affect survival rates and growth patterns. For instance, the 4:2:2 (peat:river sand:peanut shell) proportion of substrates has been associated with optimal survival and best-growth factors such as plant height and root elongation (Zhu et al., 2019; Wang et al., 2022). Similarly, peat and bark in the proportion of the 14:1:5 ratio have been highly successful, with the survival rate being as high as 98.1%3. These findings highlight the importance of careful substrate composition choice to maximize tissue culture seedling survival and growth (Figure 1).

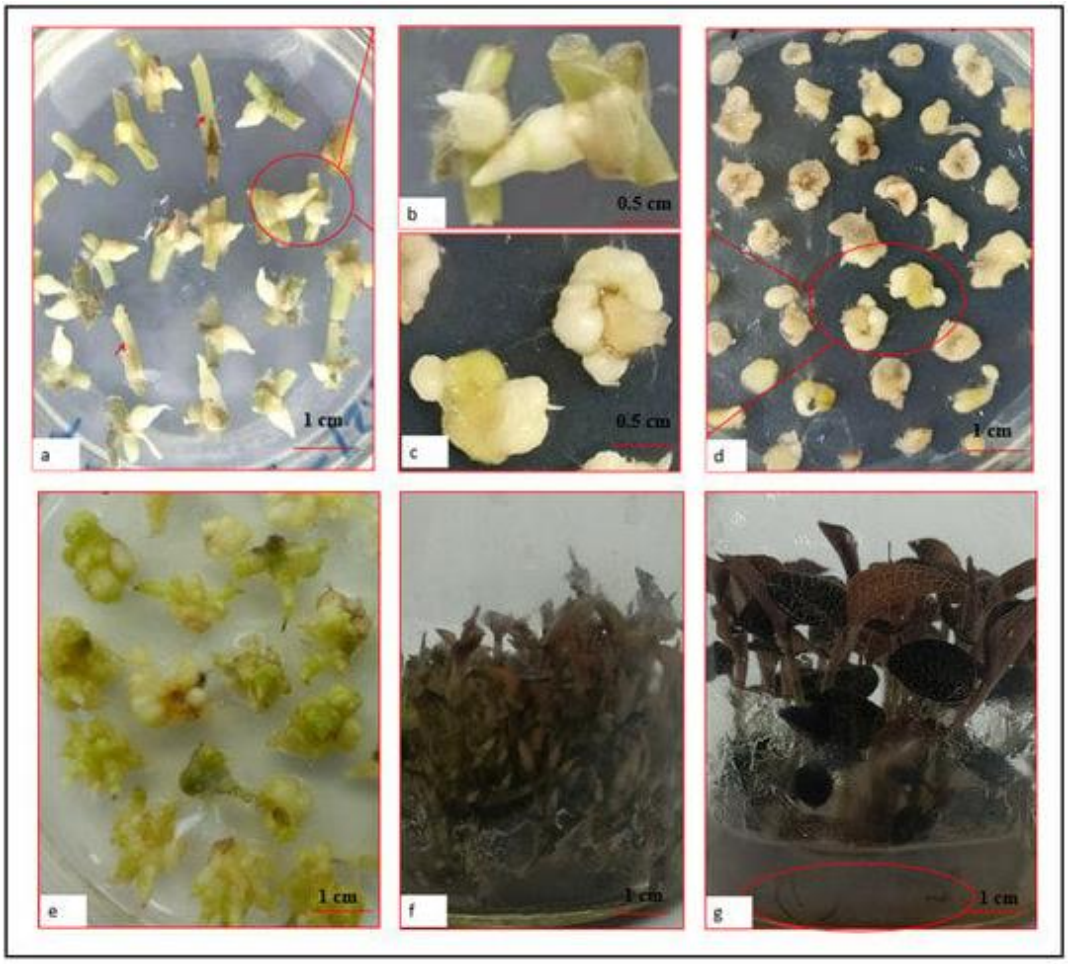


Figure 1 The effect of different matrix on the growth of *Anoectochilus roxburghii* (Adopted from Wang et al., 2022)

4 Effects of Transplanting Substrates on the Growth Quality of Tissue Culture Seedlings

4.1 Impact of different substrates on plant growth characteristics

Anoectochilus roxburghii tissue culture seedling growth behavior is greatly reliant on transplanting media selection. Research has identified that peat and river sand media at a 2:1 ratio can result in maximum survival rates and improved parameters of growth such as plant height, stem diameter, and fresh weight (Ye et al., 2020). Additionally, peat, bark, and other materials substrate composition of 14:1:5 has been reported to be optimal for better growth conditions with 98.1% survival rate and mean plant height of 9.9 cm after 90 days (Li et al., 2020). These findings specify the role of substrate composition towards achieving the maximum growth quality of *A. roxburghii* seedlings.

4.2 Differences in root growth and biomass distribution

The substrate also affects the root growth and biomass allocation of *A. roxburghii* seedlings. A 4:2:2 peat soil: river sand: peanut shell ratio has been found to enhance root growth, and the length of the longest root and the root diameter were significantly higher compared to other combinations of substrates. Such a substrate mix supports even-distributed biomass that results in better seedling vigor and overall seedling health. Moreover, with the use of a peat, muck, and vermiculite mix at a 1:1:1 proportion, there is indication that it can achieve a root rate of more than 95%, thus being effective in healthy root development (Zhang et al., 2015).

4.3 Relationship between substrates and accumulation of bioactive compounds

The composition of bioactive compounds within *A. roxburghii*, such as polysaccharides, flavonoids, and polyphenols, varies according to substrate composition. Substrate ratio 4:2:2 had no major effect on polysaccharide content, while a 4:2:1 substrate ratio was observed to increase flavonoid content in *A. formosanus*. The 4:2:2 substrate ratio was also identified as being associated with increased content of polyphenols in *A. formosanus* and *A. chapaensis*, which suggests substrate can play a role in optimizing the drug value of the plant. The findings suggest that optimizing substrate blends could not just grow more in quality but optimize the pharmacology value of *A. roxburghii* (Gam et al., 2020).

5 Screening and Optimization of Transplanting Substrates

5.1 Optimization methods and experimental design for substrate formulation

Substrate optimization of *Anoectochilus roxburghii* involves the testing of the various substrate mixtures to enhance the quality and survival rate of tissue culture seedlings. Trial experiments usually involve a change in the ratio of constituents such as peat, river sand, bark, and vermiculite. For instance, an experiment ascertained that a ratio of 2:1 of peat and river sand had optimum conditions for growth and high survival1. A single study indicated that a mix of 14:1:5 peat, bark, and other components was effective for 98.1% survival after 90 days (Gam et al., 2020). Orthogonal experiments in these cases are employed to determine the effects of different substrate components on growth parameters (Lei et al., 2017).

5.2 Comprehensive evaluation of growth quality across substrate combinations

The quality of growth of *Anoectochilus roxburghii* in various substrate combinations is measured based on parameters including stem diameter, plant height, length of the roots, and fresh weight. With a combination of peat soil, river sand, and peanut shell in the ratio of 4:2:2, maximum plant height, stem diameter, and root length were observed. In addition, bark and peat substrates were also discovered as economically suitable substrates allowing for healthy growth, with significant variation in plant growth and survival rates. Such assessment is relevant in the determination of substrates that not only stimulate plant growth but also influence active compound concentrations such as flavonoids and polyphenols (Chen et al., 2021).

5.3 Economic and environmental benefits of selecting optimal substrates

Optimum substrate choice for cultivation of *Anoectochilus roxburghii* has economic and ecological benefits. Economically, bark and peat substrates are low cost and readily available, therefore the best for large-scale production (Jin et al., 2018). Ecologically, high survival rate and plant quality are obtained through the use of substrates, hence doing away with chemical fertilizers and pesticides, therefore minimizing environmental

degradation. In addition, substrates that are compatible with sustainable cultivation manners assist in the conservation of this endangered species by reducing pressure on natural populations (Shao et al., 2014). The use of natural and biodegradable materials in substrate formulation is also within environmentally sustainable cultivation manners, which promote biodiversity and soil fertility.

6 Technical Challenges in Application and Practice

6.1 Difficulties in applying substrates to large-scale cultivation

Among the key issues in large-scale culturing of *Anoectochilus roxburghii* via substrates is the variable nature of substrate composition affecting the growth and seedling survival rate of the plants. Different substrate compositions, such as peat, river sand, and peanut shells, have been shown to significantly affect the seedling survival rate and growth quality of *A. roxburghii* (Mechergui et al., 2024). Optimum substrate ratios obtained from laboratory experiments may not be directly scalable due to differences in environmental conditions and resource availability (Han et al., 2022). It may also be challenging to maintain consistent substrate quality across large areas, which would lead to varying survival rates and growth rates.

6.2 Issues of cost, resource availability, and sustainability of substrates

Cost and availability of substrates are relevant concerns with large-scale culture of *A. roxburghii*. Substrates like peat and bark, though suitable, will not be readily available, sustainable, and cost-effective for commercial use as they are limited and expensive (Jim, 2024). Their extraction and use also involve environmental costs, which have questioned sustainability. Alternatives like utilizing locally obtained materials or creating artificial substrates can be looked into so as to overcome these drawbacks (Yu et al., 2022).

6.3 Directions for improving transplanting techniques for tissue culture seedlings

To improve the transplanting techniques of *Anoectochilus roxburghii* tissue culture seedlings, research shows improving substrate composition and environmental conditions. Experiments have proved working with particular combinations of substrates, e.g., peat, muck, and vermiculite, under controlled conditions significantly enhances survival rates and quality of growth (Jin et al., 2017). Besides, integration of the endophytic fungi technology has been reported to enhance significantly the seedling survival and vigor and been proven to be a potential option for transplanting success improvement. It is clear from studies that the role of endophytic fungi in the improvement of *A. roxburghii* seedling survival and vigor of growth is considerable. These fungi have the capability of releasing plant growth hormones, such as indole-3-acetic acid (IAA), to stimulate root branching and elongation, thereby increasing the nutrient and water uptake potential of the seedlings from the medium. This is of extreme significance during *A. roxburghii* tissue culture seedling transplantation as they are extremely sensitive to environmental differences, and a healthy root system has the capability to make them survive (Pascual et al., 2018; Ye et al., 2020) (Figure 2).

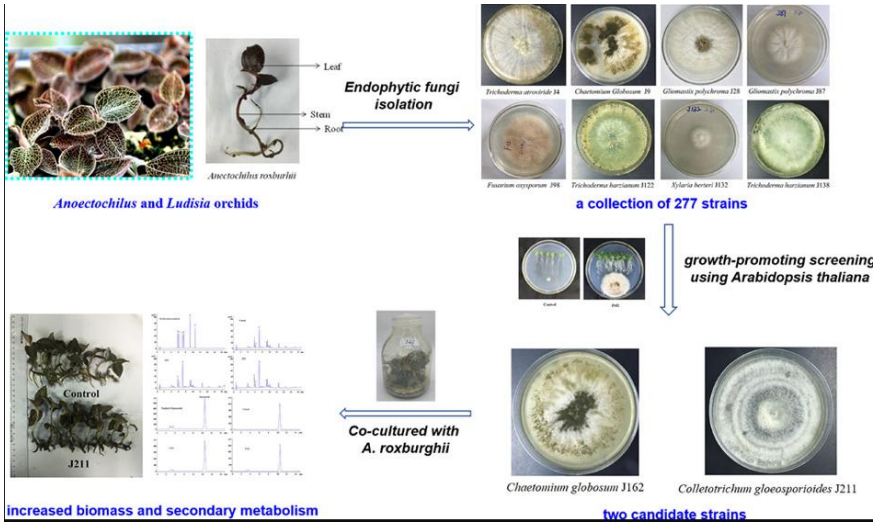


Figure 2 The Impact of Endophytic Fungi on the Transplantation of *Anoectochilus roxburghii* (Adopted from Ye et al., 2020)

7 Case Studies and Strategy Analysis

7.1 Successful cases of substrate selection for *Anoectochilus roxburghii* cultivation domestically and internationally

Optimal substrate selection has been reported in most studies for *Anoectochilus roxburghii* culture. Locally, under experiments, one study showed that the use of peat and river sand substrate mixture in a 2:1 ratio significantly improved plantlet percentage survival and quality of *A. roxburghii* plantlets with the best performance at 3 cm x 3 cm plant density and 70% shading rate (Karimi et al., 2021). Internationally, in another study, peat with bark and other material at 14:1:5 ratio was effective with an excellent survival rate of 98.1% and excellent growing conditions. Perlite blended with peat soil in the ratio of 4:1 was also found to be effective for growth of *A. roxburghii* with survival up to 97.6%.

7.2 Strategies for integrating substrate selection and cultivation management

Along with substrate choice, cultivation control includes optimizing the environment and substrate mix for improved plant development. An article recommended a peat, muck, and vermiculite mixture substrate in a 1:1:1 ratio for transplanting tissue culture plantlets in controlled environments for survival at 87.6% (Jiang., 2024). It also involves the alteration of the shade and nutrient solution concentration, as confirmed using the application of a 1/4 MS nutrient solution and 70% shading rate to result in an excellent improvement of plantlet growth and survival. In addition, there is a need for the provision of adequate environmental conditions such as temperature, pH, and humidity, as is the case with the successful culture of *A. roxburghii* under a temperature of 23°C to 28°C and relative humidity of about 90% (Ahmed et al., 2024).

7.3 Recommendations for applying substrate optimization based on this study

According to the result of this study, a substrate mixture ratio of 2 parts river sand to 1 part peat is suggested to be used in *Anoectochilus roxburghii* tissue culture seedling growth. It has been established to possess a high survival rate and improved quality growth. Furthermore, using a density of plant of 3 cm x 3 cm and a 70% shading level, additional enhancement of plantlet development can be achieved. For large-scale cultivation, a sterile blend of peat soil and sand in a ratio of 2:1 with a cover layer of live moss is suggested to achieve a high survival rate of 90.2% (Xu et al., 2017). Optimization techniques of substrate, along with proper management of the provided environment, can boost the yields of *A. roxburghii* cultivation considerably.

8 Concluding Remarks

The research on *Anoectochilus roxburghii* has substantiated that transplanting substrate selection significantly affects the quality of growth and seedling survival rate. Studies have verified that a peat and river sand mixture at a 2:1 ratio, combined with certain planting density and shading, yields high levels of survival and optimal conditions for growth. Besides, substrate mix comprising peat, bark, and plant material in the ratio 14:1:5 has been used with high success and a survival rate of 98.1% after 90 days. Incorporation of endophytic fungi into peat and vermiculite medium has also been proved to induce survival percentages as well as fresh and dry weights of the seedlings.

Even with the promising results, there are restrictions to the study. The study inclines to investigate a limited series of substrate compositions without extensive cross-experimentation of potential materials and their reactions. Additional study should also consider long-term effects of these substrates on the active compounds of *Anoectochilus roxburghii*, such as the polysaccharides and flavonoids. Future research should seek to test broader substrate compositions and long-term action of these compounds on plant growth as well as biochemical composition.

To ensure effective cultivation and industrialization of *Anoectochilus roxburghii*, optimizing the use of effective substrate combinations such as peat and river sand or peat and bark that have been previously proven to ensure high survival and healthy growth is recommended. In addition, inclusion of endophytic fungi in cultivation will further enhance plant vigor and yield. It is also essential that thorough research on the scalability and cost-effectiveness of such methods is undertaken so that they are effectively scaled up.

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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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