

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).