

3.3 Precise environmental regulation technology for *Anoectochilus roxburghii* cultivation

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

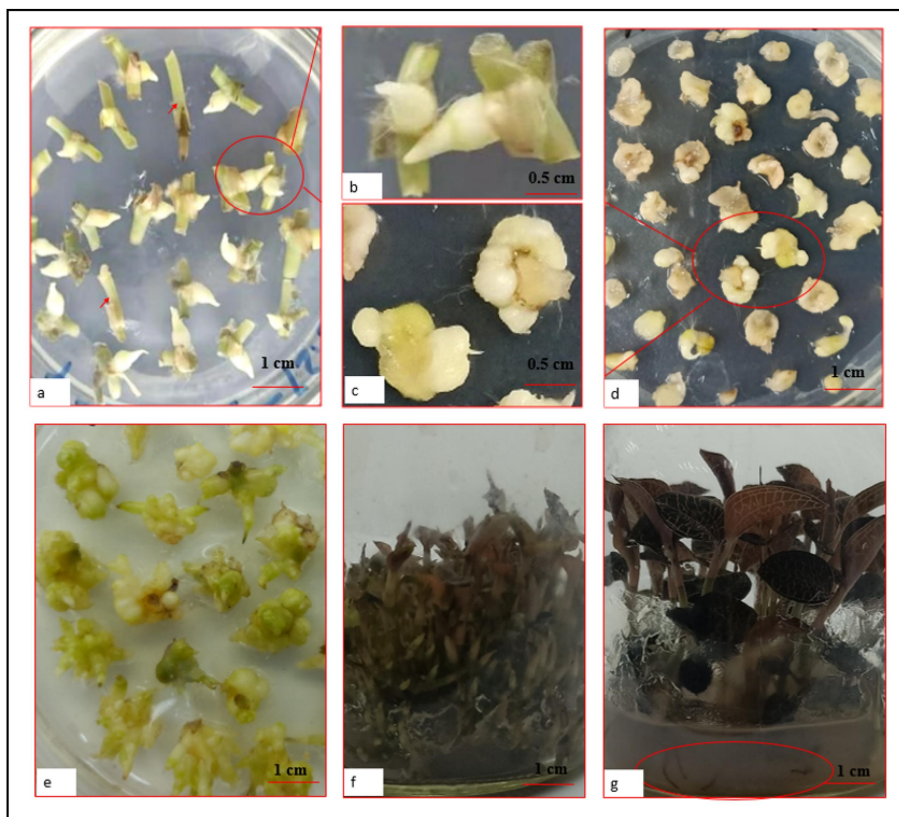


Figure 1 Induction, proliferation, and regeneration of *A. roxburghii* PLB (Adopted from Wang et al., 2022)

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

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