

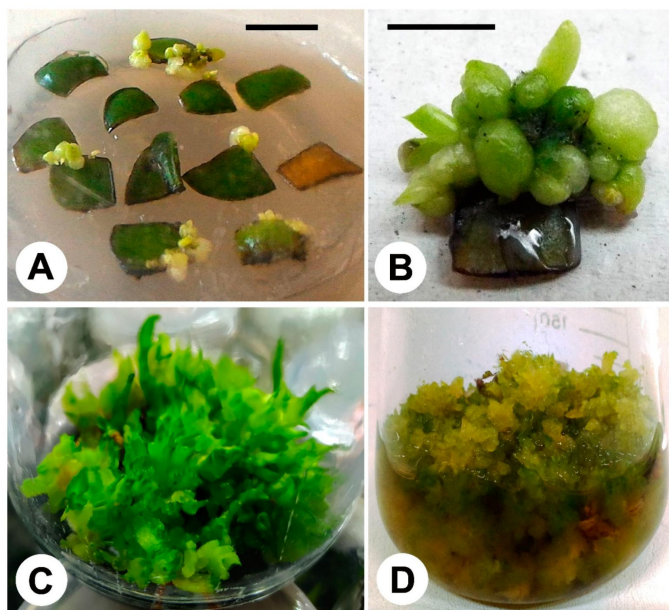


Figure 1 Induction, proliferation and regeneration of protocorm-like bodies in *Dendrobium* and *Phalaenopsis* orchids (Adopted from Cardoso et al., 2020)

Image caption: Protocorm-like bodies (PLBs)-directly induced from leaf segments of *Phalaenopsis* hybrid '501' (A) obtained from young in vitro shoots from inflorescence nodal segments and details of secondary PLBs (B) obtained in New Dogashima Medium (NDM) culture medium. Proliferation of PLBs in agar (C) and liquid (D) MS½ culture medium of *Dendrobium* 'Hybrid 3'. Bars=1 cm. Unpublished photos of Cesar A. Zanello (A,B) and Jean C. Cardoso (C,D) (Adopted from Cardoso et al., 2020)

Hormone and additive optimization remains central during proliferation culture. In a recent *D. officinale* study, 75 µM melatonin accelerated the appearance of white reticulated structures on PLB surfaces and was more conducive to PLB proliferation than the untreated control, whereas 100 µM inhibited adventitious bud differentiation (Tang et al., 2024). Cross-orchid evidence supports the need for species-specific tuning: 2.5 mg/L NAA or 0.5~2.5 mg/L BAP increased PLB percentage and fresh weight in *Dendrobium* sp., while BA with IBA or TDZ plus coconut water improved PLB proliferation and plantlet regeneration in other orchids (Hussien et al., 2024). Modified basal media can also raise PLB biomass without relying heavily on growth regulators; in *Dendrobium Sabin Blue*, a revised half-MS formula increased PLB dry mass by 23% and was proposed to reduce both production cost and the likelihood of somaclonal variation (Chin et al., 2021). Emerging approaches such as liquid shaking culture, temporary immersion systems, and oxygen nanobubbles also appear promising for improving proliferation efficiency, though direct validation in *D. officinale* remains limited (Cardoso et al., 2020; Mawardi et al., 2024).

3.3 Control of browning and vitrification

Browning is one of the main barriers to successful PLB culture because oxidation of phenolic compounds reduces regeneration capacity, suppresses growth, and can cause tissue necrosis or death (Permadi et al., 2024; Kuluev, 2020). Reviews across plant tissue culture agree on several core controls: presoaking explants in antioxidant solutions, incorporating antioxidants such as PVP or ascorbic acid into the medium, using activated charcoal, shortening subculture intervals, and imposing an initial dark treatment (Amente and Chimdessa, 2021; Permadi et al., 2024). Mechanistic evidence shows that browning is closely linked to PPO, POD, and PAL activity; vitamin C, citric acid, activated charcoal, and PVP can all suppress browning to different degrees, with vitamin C performing best in one mechanistic study by reducing PPO and POD activity and lowering total polyphenols (Xu et al., 2023).

Activated charcoal is especially relevant in orchid culture because it adsorbs inhibitory compounds, toxic metabolites, phenolic exudates, and brown exudate accumulation, although it can also adsorb vitamins and plant growth regulators, so its concentration must be optimized rather than added indiscriminately. Additional evidence from recalcitrant species shows that ascorbic acid can outperform other antioxidants in some systems, while activated charcoal at 150~200 mg/L can be better for culture establishment and shoot proliferation in others,