

high-survival transplant technology is a necessary prerequisite for the modern industrialization and high-value utilization of *Dendrobium officinale*.

2 Technical Basis of Tissue Culture Rapid Propagation of *Dendrobium officinale*

2.1 Selection of explants of *Dendrobium officinale*

The choice of explant is the first determinant of rapid propagation efficiency in *Dendrobium officinale*, because explant type affects contamination risk, induction rate, regeneration pathway, and the uniformity of resulting seedlings. Current *Dendrobium* aseptic culture protocols are strongly tissue- and genotype-dependent, so explant selection cannot be separated from the later sterilization and culture scheme (Sukmadjaja and Widhiastuti, 2019). In *D. officinale*, reported explants include seeds, stem segments with nodes, budding stems, stem tips, leaves, and protocorm-like bodies, indicating that the species has multiple available regeneration routes (Nguyen et al., 2022). For large-scale initiation, seeds are especially important because they are abundant and can directly enter asymbiotic germination and protocorm induction systems (Mamun et al., 2018).

Comparative evidence indicates that seeds are often the most suitable explant for protocorm and PLB induction in *D. officinale*. One study comparing five explant types reported a 100% induction rate and the best growth status from seeds, while leaves, stem fragments, stem tips, and stems with nodes showed lower induction rates of 43%, 25%, 52%, and 31%, respectively. However, for clonal multiplication of selected germplasm, nodal and stem-derived explants have clearer advantages because they preserve elite genotypes and support uniform vegetative propagation (Nguyen et al., 2022). Stem segments with nodes were reported to achieve 93.3% bud induction on 1/2 MS supplemented with 1.5 mg/L 6-BA and 0.5 mg/L NAA, while budding stems have also been used to establish complete rapid-propagation systems (Silva et al., 2017). Therefore, seed explants are more suitable for high-frequency initiation and PLB establishment, whereas nodal or budding-stem explants are more suitable for stable clonal propagation of target lines (Mamun et al., 2018).

2.2 Establishment of an aseptic culture system for *Dendrobium officinale*

The establishment of an aseptic culture system is the technical prerequisite for all subsequent stages of *D. officinale* micropropagation, because contamination at culture initiation directly reduces explant survival and later regeneration success. In *Dendrobium*, aseptic culture success depends on the source, size, age, and physiological state of explants, as well as the preparation and disinfection procedure used before inoculation. Most donor materials are obtained from greenhouse or similar non-sterile environments, so surface sterilization is indispensable before explants can be transferred into in vitro culture (Sukmadjaja and Widhiastuti, 2019). Because different tissues respond differently to disinfectants, no single sterilization procedure is universally optimal for all *Dendrobium* explants or genotypes.

For *D. officinale*, ethanol combined with mercuric chloride remains one of the most commonly reported effective sterilization schemes for stem-derived explants. A systematic rapid-propagation study found that 75% ethanol for 30 s followed by 0.1% HgCl₂ for 10 min was the best disinfection method for budding stems (Silva et al., 2017). Supporting evidence from another *Dendrobium* nodal-segment study showed that 0.1% HgCl₂ for 10 min produced the lowest contamination rate and the highest healthy culture establishment, while stronger sodium hypochlorite treatment increased explant mortality. In seed culture, aseptic initiation is often simplified by collecting capsules near maturity and releasing seeds directly under sterile conditions, which reduces the exposure of delicate material to harsh disinfectants. Thus, the aseptic culture system of *D. officinale* should be built around explant-specific sterilization, strict donor material selection, and careful handling during inoculation (Sukmadjaja and Widhiastuti, 2019).

2.3 Optimization of culture media and nutritional conditions for *Dendrobium officinale*

Optimization of culture media and nutritional conditions is the core of rapid propagation in *D. officinale*, because different developmental stages require different balances of mineral salts, plant growth regulators, carbon sources, activated carbon, and natural organic additives (Mamun et al., 2018). Across available studies, MS and 1/2 MS are the main basal media, but the optimal formulation varies with explant type and culture objective rather than