

weeks on sugar-free 1/2 MS subsequently achieved 96% survival after greenhouse transfer. For *D. officinale*, strong in vitro seedlings with better vigor and uniformity were obtained under moderate auxin regulation, which provides a better material basis for later acclimatization.

Ex-bottle acclimatization should then proceed by staged humidity reduction, light regulation, and use of porous, moisture-retentive substrates. Reviews of *Dendrobium* acclimatization recommend greenhouse hardening under fog or mist conditions with progressive reduction of humidity and gradual increase of light using shading management (Silva et al., 2017). Related hardening systems commonly maintain initial relative humidity near 95% with plastic covers or inverted containers, then gradually reduce moisture and nutrient supply as seedlings move to shade-house conditions (Mahendra et al., 2020). Potting mixtures successful in orchids include brick plus charcoal covered with moss and enclosed with polyethylene bags, peat-perlite-bark combinations, cocopeat, husk charcoal, and bark-based media, all of which improve aeration, drainage, and root spread during the transition period (Alghanimy and Alamery, 2025; Luthfi et al., 2026). In *D. officinale*, bark and moss-based transplant substrates were specifically identified as suitable, and one rapid propagation system further combined bark-moss substrate with gibberellin and *Epulorhiza* sp. during transplanting.

6.3 Cleaning and disinfection after bottle removal

After bottle removal, the first operation should be careful cleaning of the root system to remove residual medium, because attached medium can retain excess moisture and increase contamination risk during transplant establishment. General hardening reviews state that regenerated plantlets are usually separated completely from the MS medium and the roots are thoroughly washed before planting (Mahendra et al., 2020). This step is especially important because newly exposed roots and basal tissues are highly vulnerable to soil microorganisms during early acclimatization, and sudden exposure to microbial communities is a major cause of mortality in tissue-cultured plants (Bag et al., 2019). Accordingly, many systems use initially sterilized or disinfected substrates to reduce pathogen pressure during establishment (Bag et al., 2019). In *D. officinale* seedling domestication practice, management after bottle and subsequent field planting are treated as distinct technical links, underscoring the importance of post-bottle cleaning and handling for maintaining high survival.

Disinfection after bottle removal should balance microbial control with avoidance of phytotoxic injury. In orchid systems, chemical disinfestation with commercial bleach has been used for re-cultivation, and one study found no significant growth difference between plantlets transferred in autoclaved vessels and those managed with bleach-based disinfection (Fontes et al., 2017). However, disinfectant concentration is critical, because increasing sodium hypochlorite levels can reduce germination or increase toxicity, while lower concentrations can still control contamination. For orchid explants more broadly, effective sterilization has been reported with 70% ethanol followed by sodium hypochlorite or HgCl₂, but stronger or prolonged treatments can increase mortality (Kajol et al., 2024). Evidence from hydrogen peroxide treatments suggests that H₂O₂ is more effective against fungal than bacterial contamination, so it should not be relied on alone when bacterial pressure is high (Mtd et al., 2026). Therefore, post-bottle preparation of *D. officinale* should combine root washing, removal of damaged tissues, transplanting into clean porous substrate, and cautious use of low-to-moderate disinfection intensity to reduce contamination without compromising seedling viability.

7 Transplant Survival Improvement Techniques

7.1 Selection and formulation of transplant substrates

The transplant substrate is a primary determinant of post-bottle establishment in *Dendrobium officinale* because it controls aeration, water retention, root anchorage, and microbial exposure during the most vulnerable recovery period. Direct *D. officinale* studies identified several effective formulations, including vermiculite:perlite:humus soil at 1:1:5, which was selected as the corresponding transplant medium in an industrialized regeneration system, and bark-based matrices, with pure bark giving the highest survival in one rapid propagation study. Another *D. officinale* protocol recommended a bark-moss base combined with *Epulorhiza* sp., while substrate metabolomics work showed that pine bark, coconut coir, and their 1:1 mixture all support cultivation, but they produce different metabolic outcomes, underscoring that substrate choice affects both survival and seedling quality (Zhang et al., 2024).