

culture success, so seedlings entering this stage should first be separated by vigor and developmental completeness (Faradilla et al., 2022; Sari et al., 2023; Turnip, 2023). In *D. officinale*, reports describing improved vigor and uniformity under selected rooting treatments imply that uniform seedlings are a legitimate management target before transplanting. Studies on other orchids show that post-culture evaluation commonly records plantlet height, leaf number, root length, plant height, SPAD, NDVI, and related phenotypes during acclimatization, providing a practical basis for pre-bottle grading criteria (Mullin et al., 2022).

A practical grading system for *D. officinale* before bottle removal should therefore distinguish at least three classes: seedlings suitable for immediate transplanting, seedlings needing continued strengthening, and inferior seedlings to be discarded. Seedlings selected for removal should have a well-developed root system and balanced shoot growth, because such plantlets consistently show higher survival in later hardening studies (Poniewozik et al., 2021; Sarmah et al., 2024). Rooted orchid plantlets grown on 1/2 MS and then shifted briefly to sucrose-free medium acclimatized successfully with 96% survival in one study, suggesting that seedlings should ideally be physiologically prepared, not merely rooted, before transfer. Substrate studies further show that survival varies with media and seedling quality, with favorable outcomes reported in brick+charcoal, coconut coir+charcoal, wood sawdust, and husk-charcoal-containing systems (De Stefano et al., 2022; Sari et al., 2023; Turnip, 2023). Emerging orchid work on automated grading using image-based detection of leaf and root defects achieved F1-scores of 84.44% to 90.44%, indicating that future *D. officinale* production could combine conventional morphological grading with digital inspection for more objective seedling selection (Lin et al., 2025).

6 Acclimatization and Transplant Preparation

6.1 Effects of acclimatization on survival rate

Acclimatization directly determines the ex vitro survival of tissue-cultured *Dendrobium* plantlets because seedlings transferred abruptly from culture vessels to greenhouse conditions rapidly desiccate, wilt, and often die. Gradual adaptation to lower relative humidity and higher light improves survival by allowing plantlets to correct in vitro-induced anatomical and physiological abnormalities, including weak stomatal regulation, poor cuticle function, and incomplete autotrophic competence (Silva et al., 2017). Structural evidence from orchids further shows that hardening strengthens the cuticle, epidermis, mesophyll, and vascular tissues, and these changes were associated with 100% survival in one ex vitro hardening study (Manokari et al., 2023). For *D. officinale* specifically, domestication management during the seedling-training period raised survival during hardening to about 95%, compared with a traditional level of 70%, and post-transplant survival after training exceeded 98%.

Survival after acclimatization also depends strongly on seedling vigor, rooting quality, and biological support. *D. officinale* seedlings generally show low survival and growth after transfer from in vitro conditions, but inoculation with *Mycena dendrobii* significantly enhanced both survival and growth by increasing stress tolerance and promoting new root formation. Other *Dendrobium* studies similarly indicate that pre-hardening on sucrose-free medium or prolonged in vitro strengthening improves later survival, with reported values of 96% in *D. aqueum* and 94% in tea after enhanced in vitro hardening (Bag et al., 2019). Across orchid acclimatization trials, suitable substrates can further stabilize survival, with values of 88.33% in cocopeat, 90% in peat-perlite-bark or river-soil mixtures, and 100% in some wood sawdust or coconut coir systems, showing that acclimatization success reflects both environmental transition and substrate matching (Turnip, 2023; Alghanimy and Alamery, 2025; Luthfi et al., 2026).

6.2 In-bottle and ex-bottle acclimatization measures

In-bottle acclimatization should begin before plantlets are removed from culture vessels, because partial hardening under controlled in vitro conditions reduces transplant shock after transfer. General ex vitro establishment research shows that lowering relative humidity and increasing light intensity before bottle removal can partially harden plantlets in vitro (Mahendra et al., 2020). Prolonged culture also improves transplant readiness: extending the strengthening period increased plantlet growth and raised acclimatization survival from 46% to 94% in one micropropagation system (Bag et al., 2019). In *Dendrobium*, transfer to sucrose-free medium before potting has been used as a transitional step to promote autotrophic adjustment, and *D. aqueum* plantlets cultured for several