

4.2 Key measures for strong seedling cultivation

Strong seedling cultivation begins after shoot differentiation and requires synchronized improvement of rooting, leaf expansion, photosynthetic competence, and ex vitro adaptability. In *Dendrobium* orchids, the most common pattern is to shift from multiplication media to lower-salt or auxin-enriched rooting media, often with activated charcoal or organic additives to improve root number and root elongation. In *D. moniliforme*, MS with 0.5 mg/L NAA+0.5 mg/L IBA gave the highest rooting rate, while in *D. chryseum* 2 mg/L IBA gave the highest root number and root length and 2 mg/L NAA performed poorly, indicating that auxin type and concentration affect root quality as much as root induction itself (Liu et al., 2023; Pathak et al., 2022). In *Dendrobium* sp. derived from PLBs, 2.5 mg/L BAP+5.0 mg/L NAA+0.5 mg/L kinetin produced 6.17 roots and 5.10 cm root length, whereas a lower-NAA combination favored shoot regeneration, reinforcing the need for stage-specific rather than one-step media design.

Hardening and transplant preparation are equally important for producing strong seedlings because in vitro plantlets must shift from high humidity and photomixotrophic growth to autotrophic growth under greenhouse conditions. Reviews of *Dendrobium* acclimatization show that gradual reduction of humidity and staged increase in light improve stomatal control, root function, and survival after transfer (Silva et al., 2017). Substrate choice also has a large effect: equal-volume pine bark, turfy soil, and peanut shells performed best for *D. moniliforme*, sterilized coconut husk supported 95% survival in *D. crumenatum*, tree fern produced 94.8% survival in *D. thyrsiflorum*, and chopped coconut husk+brick+charcoal+cocopeat gave the best vegetative hardening response in *D. nobile* plantlets (Klaocheed et al., 2021; Cuc et al., 2022; Liu et al., 2023; Khilari et al., 2023). Even with optimized protocols, survival remains variable across species, ranging from 52.73% in *D. heyneanum* to 92% in *Dendrobium* 'Red Bull' and 87% in *D. heterocarpum*, so strong seedling cultivation should be judged by both vigor and post-transplant stability rather than by in vitro growth alone (Mamun et al., 2018; Longchar and Deb, 2022; Kaladharan et al., 2024).

4.3 Quality evaluation of tissue-cultured seedlings

Quality evaluation of tissue-cultured seedlings should integrate morphology, rooting status, physiological performance, acclimatization outcome, and genetic or phenotypic stability. Morphological indicators remain the most practical primary screen, including shoot height, leaf number, leaf size, root number, root length, and overall plant compactness, because these traits respond predictably to medium and environmental optimization (Khilari et al., 2023; Feng et al., 2025). Physiological quality adds another layer: in orchid seedlings, light quality alters chlorophyll, carotenoids, fresh weight, Rubisco activity, and the transition from C3 toward CAM metabolism, showing that visually similar seedlings can differ substantially in photosynthetic readiness for ex vitro growth. General tissue culture evidence also supports the use of soluble sugars, soluble proteins, and antioxidant enzyme activity as indicators linked to proliferation and seedling physiological status (Feng et al., 2025).

Final seedling quality should also be verified by performance after transplanting, because survival and normal development are the most direct tests of whether in vitro seedlings are truly strong. In *D. crumenatum*, regenerated plantlets showed 95% survival and normal greenhouse development, while *D. nobile* plantlets showed 82.3% survival together with high genetic stability by SCoT and IRAP markers, and *D. heterocarpum* showed 87% transplant survival with 96.89% monomorphism in fidelity testing (Klaocheed et al., 2021; Longchar and Deb, 2022). New image-based grading approaches in orchids further show that top-view and side-view imaging can identify root and leaf defects with F1-scores up to 90.44%, suggesting that future *D. officinale* quality evaluation can combine conventional morphological grading with automated digital inspection (Lin et al., 2025). Taken together, high-quality tissue-cultured seedlings of *D. officinale* should be defined not only by rapid in vitro growth, but by balanced shoot-root development, physiological robustness, high transplant survival, and stable true-to-type performance.

5 Rooting and Pre-Transplant Management

5.1 Optimization of rooting culture medium

The optimization of rooting culture medium in *Dendrobium officinale* should follow a stage-specific, reduced-salt, auxin-regulated strategy rather than a single universal formula. In direct studies on *D. officinale*, half-strength MS