

was repeatedly used as the basal medium for rooting, and the best reported formulations included 1/2 MS+2.5 mg/L NAA+15% potato juice+2 g/L activated carbon, which produced a rooting rate of 90.4%-92.8%, and 1/2 MS supplemented with 10% banana puree+1.0 mg/L NAA, which produced stronger roots and more vigorous plantlets. Another *D. officinale* optimization study found that two closely related media, both based on 1/2 MS with mashed potatoes, low BA, low NAA, activated carbon, Huabao No.1, and 3% sucrose, were the best overall for rooting and seedling growth promotion. Evidence from related *D. officinale* propagation systems also shows that banana-containing media are useful in the seedling-root growth stage, reinforcing the value of organic additives during rooting culture (Turnip, 2023).

Auxin type and concentration strongly affect the architecture and later usefulness of the root system, but the optimal regulator is not identical across orchid taxa. In *D. officinale*, invigorating culture showed no major vigor difference across 0~2.5 mg/L exogenous auxin overall, yet IBA at 0.75 mg/L or NAA at 0.5 mg/L improved uniformity and vigor, while NAA at 1.0 mg/L with banana puree improved root performance. In other *Dendrobium* species, NAA often increased root number whereas IBA more often promoted root elongation, although some studies reported the reverse depending on concentration and genotype (Mirani et al., 2017; Tini et al., 2025). For example, *D. aqueum* produced 8.75 roots per shoot on 1/2 MS+IBA 5 mg/L, but the longest roots were obtained with NAA 7 mg/L. By contrast, *Dendrobium* cv. Sonia Earsakul and *Phalaenopsis amabilis* both responded best to IBA at 1 mg/L for root length and rooting percentage, while *D. chrysotoxum* showed stronger rooting under relatively high IBA or NAA combined with low cytokinin levels (Sabastian et al., 2022; Alghanimy and Alamery, 2025). These comparisons indicate that for *D. officinale*, medium screening should prioritize half-strength salts, moderate auxin supply, activated carbon, and organic additives, while avoiding the assumption that more auxin always gives better roots (Seliem et al., 2020; Poniewozik et al., 2021).

5.2 Evaluation of root development quality

The evaluation of root development quality in *D. officinale* should go beyond rooting percentage and include root number, root length, uniformity, thickness, vigor, and the subsequent capacity to support acclimatization. Orchid studies consistently identify in vitro rooting as the stage most directly linked to successful transplantation, because acclimatization performance depends primarily on the roots produced before bottle removal (Mirani et al., 2017; Poniewozik et al., 2021). Accordingly, root quality should be judged as a compound trait. In *D. officinale*, the best media were described not only by rooting rate but also by stronger growth, better vigor, and more uniform seedlings. In *D. anosmum*, activated charcoal-containing medium improved shoot length, leaf number, root number, and root length simultaneously, illustrating that a usable transplantable seedling requires balanced whole-plant quality rather than roots assessed in isolation (Nguyen et al., 2022).

Cross-species evidence helps clarify which root traits are most meaningful for pre-transplant assessment. In *D. nobile*, NAA alone outperformed IBA alone for root number, length, and thickness, but combined NAA+IBA reduced rooting and induced callus at the shoot base, showing that visually abundant rooting is not necessarily desirable if root bases are abnormal (Mirani et al., 2017). In *Paphiopedilum insigne*, 1 mg/dm³ IAA or IBA produced many rooted, good-quality plantlets, and the best later acclimatization followed in vitro rooting treatment plus a suitable substrate, indicating that root quality must be validated by ex vitro performance (Poniewozik et al., 2021). In *Phalaenopsis*, nano-selenium plus 0.5 mg/L NAA increased rooting percentage, root length, and root number and then improved acclimatization, again linking root metrics to transplant readiness (Seliem et al., 2020). Practical evaluation in *D. officinale* should therefore classify high-quality roots as numerous enough for anchorage, sufficiently elongated, evenly distributed, free of basal callus or browning, and associated with vigorous leaves and stems, because these combined features are the best predictors of survival after transfer (Mirani et al., 2017; De Stefano et al., 2022).

5.3 Grading of seedlings before bottle removal

Seedlings should be graded before bottle removal because pre-transplant selection improves the consistency of acclimatization management and reduces losses from weak or non-uniform plantlets. The acclimatization literature treats transfer from bottle to external substrate as a critical stage and an important indicator of tissue