

reinforcing that anti-browning recipes remain species-dependent (Guntur et al., 2019; Jakhar et al., 2019). Direct vitrification evidence is sparse in the supplied *D. officinale* corpus, but practical control still follows the same logic as browning prevention: avoid prolonged exposure to excessively high cytokinin levels, maintain moderate inoculum density, and use timely subculture to prevent water-soaked, hyperhydric tissues during rapid proliferation (Fan et al., 2016; Tang et al., 2024). Overall, effective PLB culture in *D. officinale* requires coupling high-proliferation conditions with antioxidant management and disciplined passage schedules, so that rapid multiplication does not come at the cost of tissue quality.

4 Multiple Shoot Induction and Strong Seedling Cultivation

4.1 Regulation of multiple shoot differentiation

The regulation of multiple shoot differentiation in *Dendrobium officinale* depends primarily on the coordinated adjustment of basal medium, cytokinin-auxin balance, and the developmental state of the cultured material. Across *Dendrobium* systems, shoot formation is mainly driven by plant growth regulator composition rather than by any single additive alone, and successful regeneration generally requires species-specific optimization (Pasternak and Steinmacher, 2024; Erkoyuncu, 2026). In related *Dendrobium* species, BA or BAP combined with low to moderate NAA repeatedly promoted shoot proliferation, including MS+1.0 mg/L BA+1.0 mg/L NAA in *D. moniliforme* with a 6.1-fold cluster-shoot proliferation coefficient, MS+3.0 mg/L BAP+1.0 mg/L NAA in *Dendrobium* ‘Red Bull’ with 7.66 shoots per explant, and MS+0.5 mg/L BAP+0.5 mg/L NAA in *D. chryseum* with 5.8 shoots per explant (Mamun et al., 2018; Pathak et al., 2022; Liu et al., 2023). These findings are consistent with broader orchid evidence that cytokinin-dominant media promote shoot induction, while excess auxin tends to redirect development toward callus or rooting rather than repeated shoot differentiation (Kaladharan et al., 2024).

For protocorm-like body and protocorm-derived cultures, multiple shoot differentiation is also strongly influenced by organic supplements and activated charcoal. In *D. crumenatum*, MS+15% coconut water produced 96.0% shooting and 9.5 shoots per explant, while activated charcoal further improved leaf and root development, showing that shoot induction and seedling quality can be uncoupled and optimized sequentially (Klaosheed et al., 2021). Similar responses were reported in *D. thyrsiflorum*, where 0.4 mg/L BA+0.4 mg/L kinetin gave the highest multiplication rate of 4.53 times, and in *D. heyneanum*, where 1.0 mg/L kinetin produced the highest protocorm-derived micropropagation frequency of 90.20% (Figure 2) (Cuc et al., 2022; Kaladharan et al., 2024). Additional evidence from general orchid tissue culture indicates that light quality modifies shoot proliferation by altering sugar accumulation, chlorophyll synthesis, and antioxidant activity, but the response is species-specific, so light should be treated as a secondary regulatory factor after the hormone regime is fixed (Mehbub et al., 2022; Feng et al., 2025).

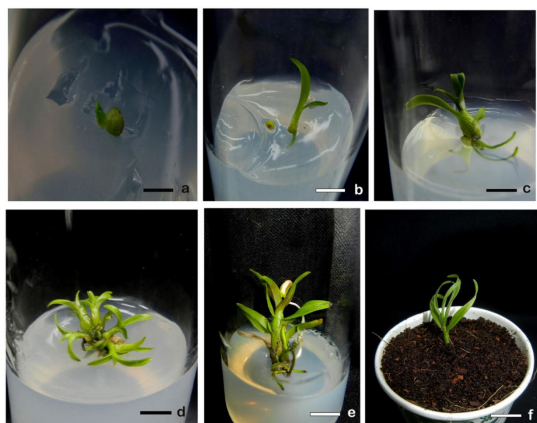


Figure 2 Micropropagation of *Dendrobium heyneanum* Lindl. from protocorms (Adopted from Kaladharan et al., 2024)
Image caption: a) Protocorm (Stage IV); b) Seedling formation from protocorms; c) Shoot with developing pseudobulb and roots; d) Multiple shoot bud formation; e) Elongation of Pseudobulb and roots; f) Hardened plantlet (Adopted from Kaladharan et al., 2024)