

following a single universal recipe (Silva et al., 2017). For example, 1/2 MS without phytohormones was reported as optimal for aseptic seed germination, whereas protocorm induction and proliferation were improved on MS supplemented with 1.0 mg/L 6-BA, 1.0 mg/L NAA, and 1.0 mg/L KT (Mehbub et al., 2022). In another seed-based system, the most suitable germination medium was 1/2 MS+0.2 mg/L NAA+100 g/L potato+25 g/L sucrose, while the most suitable rooting and seedling medium was 1/2 MS+0.2 mg/L NAA+100 g/L banana+25 g/L sucrose+0.5 g/L activated carbon (Mamun et al., 2018).

For stem- and node-derived explants, cytokinin-auxin combinations are central to shoot induction and multiplication. In *D. officinale*, effective formulations include MS+1.0 mg/L 6-BA+0.5 mg/L NAA+2 g/L activated carbon for budding stem induction, MS+0.5 mg/L 6-BA+0.8 mg/L 2,4-D+2 g/L activated carbon for proliferation, and 1/2 MS+1.5 mg/L 6-BA+0.5 mg/L NAA for both bud induction and multiple-shoot culture (Mamun et al., 2018). Other studies likewise reported that MS+2.0 mg/L 6-BA+0.1 mg/L NAA gave the highest cluster-shoot proliferation, and that coconut milk, coconut juice, potato extract, banana extract, and activated carbon could further promote PLB propagation, seedling growth, or rooting (Silva et al., 2017; Nguyen et al., 2022). Rooting media generally shift toward reduced salt concentration and stronger auxin support, such as 1/2 MS+2.5 mg/L NAA+15% potato juice+2 g/L activated carbon, MS+0.5 mg/L IBA+0.5 mg/L NAA, or 1/2 MS+0.2 mg/L IBA+0.5% activated carbon+40% potato extract (Mehbub et al., 2022). Rapid propagation of *D. officinale* depends on a stage-specific medium optimization strategy rather than a single fixed medium, and this staged regulation is the basis for high regeneration efficiency and subsequent strong seedling formation.

3 Protocorm Induction and Proliferation Culture

3.1 Conditions for protocorm induction

Protocorm-like body induction in *Dendrobium officinale* depends first on a suitable basal medium and a balanced cytokinin-auxin combination. A targeted study using plumules as explants identified 1/2 MS supplemented with 2% sucrose, 10% banana puree, pH 6.0, plus 6-BA 0.3 mg/L and NAA 0.2 mg/L as the optimal induction condition for PLBs (Fan et al., 2016). More broadly in *Dendrobium* orchids, auxins and cytokinins are the growth regulators most commonly used for PLB induction, and PLBs themselves are considered the most responsive explant type for propagation systems (Arli et al., 2023). Comparative orchid evidence also shows that induction response varies strongly by genotype and regulator type: thidiazuron can be highly effective in some *Dendrobium* systems, while 2iP, meta-topolins, or BA-NAA combinations outperform alternatives in others, so induction formulas should be treated as species- and material-specific rather than universal (Figure 1) (Cardoso et al., 2020).

Induction efficiency is also shaped by explant condition and culture environment after inoculation. In *D. officinale*, light-yellow, loose, and plump PLBs proliferated faster than compact or physiologically aged material, indicating that early visual screening of PLB quality improves subsequent culture performance (Fan et al., 2016). Thin Cell Layer approaches in *Dendrobium* have been reported to outperform larger conventional explants because thinner tissues improve contact with the medium and diffusion into the explant, which is relevant when designing highly responsive induction systems (Arli et al., 2023). At the developmental level, PLBs in *D. officinale* initially show embryoid-like morphology and later shift toward organogenesis, with most protocorms beginning germination and differentiation after about 35 days, so induction protocols should align subculture timing with this transition (Tang et al., 2024).

3.2 Subculture proliferation technology

After induction, subculture proliferation should prioritize physiological uniformity, inoculum density, and transfer interval. In *D. officinale*, inoculating ten PLBs with similar physiological status as one group gave better proliferation performance, and the most significant biomass gain occurred after 45 days of culture, when the proliferation rate reached 1008% (Fan et al., 2016). The same study found that laminated culture favored the upper layer for proliferation and weight gain, and also helped rejuvenate PLBs, suggesting that spatial arrangement in the culture vessel can affect oxygen, light, and nutrient access during repeated passage (Fan et al., 2016). These findings support a practical subculture strategy of selecting vigorous, light-colored PLBs, standardizing inoculum size, and transferring at roughly 45-day intervals before visible decline in vigor (Fan et al., 2016).