Indian J. Genet. Plant Breed., (2025); 85(4 Suppl.) : 813-815
https://doi.org/10.31742/ISGPB.85.suppl.15
ISSN: 0975-6906
www.isgpb.org

SHORT RESEARCH ARTICLE

Photoperiodism and plant growth regulators: optimized callus induction and regeneration in *Dendrobium orchid* var. earsakul

Khaling Lallemmoi, Sunil Kumar*, Amit Kumar Singh[1], Siddhartha Singh[1], Kalkame Ch. Momin

Abstract

Orchids are highly valued for their beauty, diversity, and commercial importance. This study examines Dendrobium Orchid var. Earsakul, a unique mutant with significant conservation and market value, to develop faster and more efficient propagation methods than conventional techniques. Tissue culture experiments were conducted using MS medium with different combinations of plant growth regulators under varied light conditions. Kinetin with NAA produced the fastest callus induction (4 days) and highest callus biomass, while light significantly enhanced callus formation compared to darkness. For shoot regeneration, BAP with NAA resulted in the earliest shoot initiation, highest shoot number, and better shoot growth. Rooting was most effective with BAP and IBA, giving early root initiation and maximum root length. Overall, the study demonstrates the crucial interaction between light and hormones in improving micropropagation and supporting conservation and commercial production of Dendrobium Earsakul.

Keywords: Dendrobium Orchid, photomorphogenesis, plant growth regulators, tissue culture, callus induction, orchid conservation

Orchids represent one of the largest and most diverse families of flowering plants, comprising nearly 22,500-35,000 species across 779 genera, and show exceptional adaptability to varied ecological conditions (Monalisha et al. 2017; Droissart et al. 2023). They contribute over 10% of global flowering plant diversity and nearly 10% of the international floriculture trade through continuous hybrid development (De and Medhi 2015). India hosts more than 1,350 orchid species, with about 900 species, including 150 endemics, concentrated in the North-Eastern region (De and Singh 2015). *Dendrobium* is a commercially important genus valued for floral quality (Leitch et al., 2009). However, *Dendrobium* var. Earsakul faces propagation challenges due to keiki-induced variability (Li et al., 2013) and conservation concerns under CITES Appendix II (Wraith and Pickering 2018). Tissue culture, regulated by photoperiodism and plant growth regulators, provides an efficient approach for propagation and conservation.

Young shoot explants of *Dendrobium* Orchid var. *Earsakul* were collected and surface-sterilized through sequential chemical treatments, and sectioned into 0.3–0.4 cm segments. Explants were cultured on Murashige and Skoog medium supplemented with 3% sucrose (pH 5.8) and agar (Murashige and Skoog 1962) and maintained at 24°C under a 16/8 h light/dark photoperiod. Various auxin-cytokinin combinations were evaluated for callus, shoot, and root induction using hormone-free medium as control.

College of Horticulture and Forestry, Pasighat, Central Agricultural University, Imphal

Department of Floriculture and Landscaping, College of Horticulture & Forestry, Central Agricultural University, Pasighat 791 102, Arunachal Pradesh, India

[1]Department of Basic Sciences and Humanities, College of Horticulture & Forestry, Central Agricultural University, Pasighat 791 102, Arunachal Pradesh, India

***Corresponding Author:** Sunil Kumar, Department of Floriculture and Landscaping, College of Horticulture & Forestry, Central Agricultural University, Pasighat 791 102, Arunachal Pradesh, India, E-Mail: sunu159@gmail.com

How to cite this article: Lallemmoi K., Kumar S., Singh A.K., Singh S. and Momin K.C. 2025. Photoperiodism and plant growth regulators: optimized callus induction and regeneration in *Dendrobium orchid* var. earsakul. Indian J. Genet. Plant Breed., **85**(4 Suppl.): 813-815.

Source of support: Nil

Conflict of interest: None.

Received: July 2026 **Revised:** Sept. 2026 **Accepted:** Oct. 2025

Rooted plantlets were acclimatized in cocopeat, perlite, and sphagnum moss (2:1:1).

Callus induction and shoot regeneration

The study showed that callus, shoot, and root development in *Dendrobium* Orchid var. *Earsakul* are strongly influenced

by photoperiod and plant growth regulators, with hormone-supplemented media essential for effective callus induction. The shortest callus induction period was observed on MS medium supplemented with 0.25 mg L^{-1} kinetin (KIN) and 5 mg L^{-1} Napthalene acetic acid (NAA) under a 16/8 h light/dark photoperiod, whereas the longest induction time was recorded in the control. Light conditions consistently promoted faster callus initiation compared to complete darkness, indicating the stimulatory role of photomorphogenic signals in early cellular activation. Similar enhancement of callogenesis under light has been reported earlier, where balanced cytokinin–auxin combinations promoted rapid cell division and morphogenesis (Maridass et al. 2010; Siddique and Islam 2015). Callus induction rate and biomass accumulation further supported the superiority of light conditions. Over 65% callus induction was achieved on media containing KIN and higher concentrations of NAA under light, while dark-grown cultures showed significantly reduced induction percentages. The maximum callus fresh weight was recorded with 0.5 mg L^{-1} KIN and 7.5 mg L^{-1} NAA, particularly under light conditions, suggesting enhanced metabolic activity and nutrient utilization. Reduced callus growth in darkness may be attributed to limited activation of light-responsive pathways involved in hormone signaling and auxin transport, as previously suggested in orchid tissue culture studies (De and Medhi 2015; Siddique and Islam 2015).

Shoot regeneration responses varied significantly with different BAP and NAA combinations. The earliest shoot initiation and highest shoot number were obtained on MS medium supplemented with 3 mg L^{-1} BAP and 0.6 mg L^{-1} NAA, highlighting the synergistic effect of cytokinins and low auxin concentrations in promoting shoot meristem differentiation. Progressive increases in shoot number and length over time further confirmed sustained organogenic competence under optimized PGR regimes. These findings are in agreement with earlier reports indicating that cytokinins play a dominant role in shoot proliferation, while auxins maintain morphogenic balance (Li et al. 2013).

Root induction in *Dendrobium* Orchid var. Earsakul was most effectively achieved using combinations of BAP and IBA, with the earliest root initiation and maximum root length observed on MS medium supplemented with 0.5 mg L^{-1} BAP and 1 mg L^{-1} IBA. IBA played a crucial role in enhancing rhizogenesis, producing longer, healthier, and more robust roots, particularly with extended culture duration. In contrast, delayed or poor rooting in hormone-free medium clearly highlighted the necessity of auxin supplementation for successful root development. Regenerated plantlets exhibited more than 70% survival during acclimatization (Fig. 1) when transferred to a substrate composed of cocopeat, perlite, and sphagnum moss, demonstrating the effectiveness and practical applicability of the protocol. This high survival rate indicates strong physiological competence and well-developed root systems in in vitro–raised plantlets. Considering the conservation significance of *Dendrobium* species under CITES Appendix II, the optimized protocol provides a reliable tool for large-scale propagation and genetic conservation (Wraith and Pickering 2018). The findings further emphasize the critical role of photoperiodism and precise PGR combinations in regulating morphogenesis, supporting efficient micropropagation, conservation, and commercial floriculture applications.

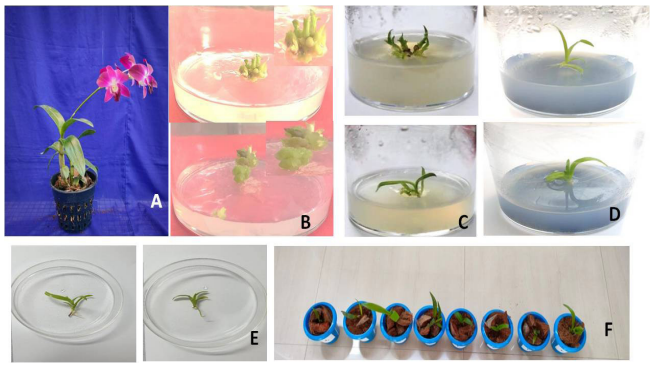

Fig. 1. In vitro regeneration stages of *Dendrobium* Orchid var. Earsakul: (A) mother plant, (B) callus formation, (C) shoot regeneration, (D) shoots on rooting medium, (E) rooted plantlets, and (F) hardened plants.

Authors' contribution

Conceptualization of research (SK, AKS, KL); Designing of the experiments (SK, AKS); Contribution of experimental materials (SK, SS, KCM); Execution of field/lab experiments and data collection (KL, SK, AKS); Analysis of data and interpretation (SK, KL, AKS, SS, KCM); Preparation of the manuscript (KL. SK, AKS, SS).

Acknowledgement

Authors are thankful to Vice Chancellor, Central Agricultural University, Imphal for providing necessary facilities and financial assistance.

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