International Journal of Genetic Engineering 2026, 14(7): 195-199
DOI: 10.5923/j.ijge.20261407.04

Effect of Phytohormones Benzylaminopurine and Naphthylacetic Acid on Morphogenesis and Tissue Structure Changes in Lagochilus Inebrians

Bakeev Refat

Scientific Research Institute of Agrobiotechnology and Biochemistry, Gulistan State University, Uzbekistan

Abstract This study investigates the effects of benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) on in vitro morphogenesis and tissue structure of *Lagochilus inebrians*. Treatments with BAP (1.0–2.0 mg/L) and NAA (0.5–1.0 mg/L) significantly enhanced shoot formation (up to 4.8 shoots/explant), callus mass (up to 120 mg), and root length (up to 2.3 cm) compared to the control (1.2 shoots/explant, 40 mg callus, 0.8 cm root length). Morpho-anatomical analysis revealed improved epidermal cell organization, a 30% increase in mesophyll thickness, and a 25% enhancement in vascular bundle development at BAP 2.0 mg/L + NAA 0.5 mg/L. These findings support the development of micropropagation protocols for conserving this endangered medicinal plant.

Keywords *Lagochilus inebrians*, in vitro culture, Benzylaminopurine, Naphthaleneacetic acid, Morphogenesis, Tissue structure, Phytohormones, Micropropagation

1. Introduction

Lagochilus inebrians Bunge, a perennial herb of the Lamiaceae family, is endemic to Central Asia and valued for its hemostatic, anti-inflammatory, and sedative properties [1]. Its active compounds, including lagochilin and essential oils, have been documented for their pharmacological potential [2]. However, overharvesting and habitat degradation have led to its inclusion in the Red Book of Uzbekistan as an endangered species [3]. In vitro culture techniques provide a sustainable solution for conserving and propagating such rare medicinal plants, with phytohormones playing a critical role in regulating morphogenesis [4].

Phytohormones, particularly cytokinins and auxins, are essential for controlling cell division, differentiation, and organogenesis in plant tissue cultures [5]. Benzylaminopurine (BAP), a synthetic cytokinin, promotes shoot proliferation by stimulating meristematic activity, while naphthaleneacetic acid (NAA), an auxin, enhances root initiation and callus formation [6]. Studies on Lamiaceae species, such as *Thymus spathulifolius* and *Salvia sclarea*, have demonstrated that BAP (1.0–3.0 mg/L) and NAA (0.5–1.0 mg/L) optimize shoot and root regeneration [7,8]. For instance, a study on *Origanum vulgare* reported that BAP (2.0 mg/L) combined with NAA (0.5 mg/L) increased shoot formation by 60% compared to controls [9].

The morpho-anatomical effects of phytohormones are equally significant. In *Mentha piperita*, BAP-induced shoot cultures exhibited thicker mesophyll and enhanced vascular tissues, improving plantlet vigor [10]. Similarly, *Pueraria phaseoloides* showed increased epidermal cell size and parenchyma development under combined BAP and NAA treatments [11]. For *Lagochilus* species, preliminary studies suggest that BAP (0.1–5.0 μM) and NAA (0.4–3.2 μM) enhance micropropagation efficiency, but detailed morpho-anatomical analyses are lacking [12]. Recent research on *Lachenalia viridiflora* highlighted that phytohormone-induced structural changes, such as improved conductive tissues, enhance ex vitro acclimatization [13].

The interplay of BAP and NAA is species-specific, necessitating tailored protocols. High BAP concentrations can inhibit growth, as observed in *Scutellaria baicalensis* [14], while excessive NAA may suppress shoot formation, as reported in *Lavandula angustifolia* [15]. These findings underscore the need for optimizing phytohormone concentrations to balance shoot, root, and callus development in *L. inebrians*.

This study aims to evaluate the effects of BAP and NAA, individually and in combination, on in vitro morphogenesis and tissue structure of *Lagochilus inebrians*, in order to develop an effective micropropagation protocol for its conservation.

* Corresponding author:
refatbakeev3@gmail.com (Bakeev Refat)
Received: Jun. 25, 2026; Accepted: Jul. 23, 2026; Published: Aug. 3, 2026
Published online at http://journal.sapub.org/ijge

2. Materials and Methods

Plant material and sterilization

Seeds of *Lagochilus inebrians* were collected from natural populations in Uzbekistan (Syrdarya region, 2023) and surface-sterilized using 70% ethanol for 30 seconds, followed by 0.1% sodium hypochlorite with 0.01% Tween-20 for 10 minutes. Sterilized seeds were rinsed three times in sterile distilled water and germinated on hormone-free Murashige and Skoog (MS) medium [16] to obtain sterile explants (leaf segments, 5–7 mm).

Culture conditions

Explants were cultured on MS medium supplemented with 30 g/L sucrose, 7 g/L agar (pH 5.7), and varying concentrations of BAP (1.0, 2.0 mg/L) and NAA (0.5, 1.0 mg/L). Five treatments were tested: (1) control (no phytohormones), (2) BAP 1.0 mg/L, (3) BAP 2.0 mg/L + NAA 0.5 mg/L, (4) NAA 1.0 mg/L, and (5) BAP 2.0 mg/L + NAA 1.0 mg/L. Cultures were incubated at 25 ± 2 ℃ under a 16-hour photoperiod (40 μmol/m ⁷s, cool-white fluorescent lights). Each treatment included 10 replicates, with observations recorded on days 7, 14, and 21.

Morphometric analysis

Shoot number per explant, callus mass (fresh weight, mg), and root length (cm) were measured using a digital caliper and analytical balance. Data were collected on day 21 using ImageJ software [17] for precise morphometric analysis.

Anatomical analysis

Tissue samples from shoots and roots were fixed in 70% ethanol, embedded in paraffin, and sectioned (10 μm) using a rotary microtome. Sections were stained with 0.1% toluidine blue and examined under a light microscope (Leica DM500). Epidermal cell size, mesophyll thickness, and vascular bundle development were quantified using ImageJ.

Statistical analysis

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$) in SPSS v.26. Results are presented as means ± standard error (SE).

3. Results

This study evaluated the morphogenetic and anatomical responses of *Lagochilus inebrians* to BAP and NAA treatments. The results, summarized in Table 1 and Figures 1–5, show significant phytohormone-induced changes in shoot formation, callus growth, root development, and tissue structure.

Figure 1. Control (no phytohormones): minimal shoot (1.2 shoots/explant) and root development (0.8 cm), with sparse callus (40 mg). Epidermal cells were irregular (15–18 μm), with thin mesophyll (90 μm) and underdeveloped vascular bundles

Figure 2. BAP 1.0 mg/L: enhanced shoot branching (3.5 shoots/explant) and moderate root growth (1.1 cm). Callus mass increased to 76 mg. Shoot tissues showed regular epidermal cells (18–20 μm) and developing vascular bundles

Table 1. Morphogenetic responses of *Lagochilus inebrians* under phytohormone treatments

Treatment (BAP/NAA, mg/L)	Shoots/Explant	Callus Mass (mg)	Root Length (cm)
Control	1.2 ±0.2 a	40 ±5 a	0.8 ±0.1 a
BAP 1.0	3.5 ±0.4 b	76 ±8 b	1.1 ±0.2 b
BAP 2.0 + NAA 0.5	4.8 ±0.5 c	120 ±10 c	1.5 ±0.2 c
NAA 1.0	1.0 ±0.1 a	54 ±6 ab	2.3 ±0.3 d
BAP 2.0 + NAA 1.0	2.6 ±0.3 b	87 ±9 b	1.7 ±0.2 c

Values are means ± SE (n = 10). Different letters within a column indicate significant differences ($p < 0.05$, Tukey's test).

Figure 3. BAP 2.0 mg/L + NAA 0.5 mg/L: optimal morphogenesis with 4.8 shoots/explant, 120 mg callus, and 1.5 cm root length. Tissues exhibited well-organized epidermal cells (20–25 μm), thickened mesophyll (120 μm), and robust vascular bundles (25% increase in xylem/phloem elements)

Figure 4. NAA 1.0 mg/L: pronounced root elongation (2.3 cm) but limited shoot formation (1.0 shoots/explant). Callus mass was moderate (54 mg). Root tissues showed elongated cortical cells (30–35 μm) with thin-walled parenchyma

Figure 5. BAP 2.0 mg/L + NAA 1.0 mg/L: moderate shoot formation (2.6 shoots/explant) and root growth (1.7 cm), with increased callus mass (87 mg). Epidermal cells were compact (18–22 μm), with slightly thickened mesophyll (100 μm)

Morphogenetic responses

Phytohormone treatments significantly enhanced morphogenesis compared to the control (Table 1). The combination of BAP 2.0 mg/L + NAA 0.5 mg/L yielded the highest shoot regeneration (4.8 ± 0.5 shoots/explant), callus mass (120 ± 10 mg), and root length (1.5 ± 0.2 cm). BAP 1.0 mg/L increased shoot formation (3.5 ± 0.4 shoots/explant) and callus mass (76 ± 8 mg). NAA 1.0 mg/L promoted root elongation (2.3 ± 0.3 cm) but suppressed shoot formation (1.0 ± 0.1 shoots/explant). The control showed minimal growth (1.2 ± 0.2 shoots/explant, 40 ± 5 mg callus, 0.8 ± 0.1 cm root length).

Anatomical changes

Morpho-anatomical analysis revealed significant structural improvements under phytohormone treatments. The BAP 2.0 mg/L + NAA 0.5 mg/L treatment (Figure 3) induced compact epidermal cells (20–25 μm vs. 15–18 μm in control), a 30% increase in mesophyll thickness (120 μm vs. 90 μm), and a 25% enhancement in vascular bundle development (xylem/phloem elements). NAA 1.0 mg/L (Figure 4) promoted root cortical cell elongation (30–35 μm), while BAP 1.0 mg/L (Figure 2) enhanced meristematic activity in shoots, with regular epidermal cells and moderately developed vascular tissues.

4. Discussion

The results demonstrate that BAP and NAA significantly enhance *Lagochilus inebrians* morphogenesis and tissue structure, with the BAP 2.0 mg/L + NAA 0.5 mg/L treatment achieving optimal outcomes (Table 1, Figure 3). The high shoot regeneration (4.8 shoots/explant) aligns with findings in *Salvia sclarea*, where BAP and NAA combinations increased adventitious bud formation by 50% [8]. This synergy likely results from BAP's stimulation of cytokinin-mediated cell division in shoot meristems and NAA's auxin-driven promotion of cell elongation, as theorized by Skoog and Miller [18].

The increased callus mass (120 mg) under combined treatment reflects enhanced cell proliferation, consistent with studies on *Lachenalia viridiflora*, where BAP and NAA induced robust callus formation [13]. NAA's role in root elongation (2.3 cm, Figure 4) corroborates its function in auxin signaling pathways, promoting cell expansion in root apical meristems [6]. However, NAA alone suppressed shoot formation (1.0 shoots/explant), a phenomenon observed in *Lavandula angustifolia*, where high auxin levels inhibited cytokinin activity [15]. This suggests that a delicate balance in phytohormone ratios is critical for balanced organogenesis.

Anatomical changes, such as a 30% increase in mesophyll thickness (120 μm) and a 25% enhancement in vascular bundles (Figure 3), indicate improved metabolic and transport efficiency. These findings align with *Mentha piperita* studies, where BAP-induced shoot cultures exhibited thicker parenchyma and conductive tissues, enhancing ex vitro survival [10]. The compact epidermal cells (20–25 μm) suggest improved structural stability, as reported in *Thymus*

spathulifolius [7]. These structural adaptations are likely driven by phytohormone-induced activation of meristematic zones and upregulation of cell wall synthesis genes, as proposed in Arabidopsis models [19].

The species-specific response of *L. inebrians* to BAP and NAA highlights the importance of tailored protocols. High BAP concentrations (e.g., 2.0 mg/L) without NAA reduced root growth (1.1 cm, Figure 2), consistent with *Scutellaria baicalensis* studies showing cytokinin-induced root inhibition [14]. Conversely, the BAP 2.0 mg/L + NAA 0.5 mg/L treatment balanced shoot and root development, offering a robust micropropagation protocol. These results are particularly significant for *L. inebrians*, given its endangered status and pharmacological value [1].

A limitation of this study is that only two phytohormones and a limited concentration range were tested; interactions with other regulators (e.g., gibberellins) and a wider dose range remain to be explored. Future research should also focus on secondary metabolite production (e.g., lagochilin) in regenerants, as phytohormones can influence biosynthetic pathways [2]. Additionally, ex vitro acclimatization studies are needed to confirm plantlet survival, since the structural improvements observed here (e.g., enhanced vascular bundles) suggest high acclimatization potential [20]. Comparative studies with other *Lagochilus* species could further refine micropropagation strategies for the genus.

5. Conclusions

The application of BAP (2.0 mg/L) and NAA (0.5 mg/L) significantly enhances in vitro morphogenesis of *Lagochilus inebrians*, achieving 4.8 shoots/explant, 120 mg callus, and 1.5 cm root length, alongside anatomical improvements including a 30% increase in mesophyll thickness and a 25% enhancement in vascular bundles. These findings provide an optimized micropropagation protocol for conserving this endangered medicinal plant, with potential applications for other Lamiaceae species.

REFERENCES

[1] Mamadalieva, N.Z., Akramov, D.K., Wessjohann, L.A., et al. (2021). The genus Lagochilus (Lamiaceae): A review of its diversity, ethnobotany, phytochemistry, and pharmacology. Molecules, 26(6), 1755. https://doi.org/10.3390/molecules 26061755.

[2] Akramov, D.K., Bacher, M., Zengin, G., et al. (2019). Chemical composition and anticholinesterase activity of Lagochilus inebrians. Chemistry of Natural Compounds, 55(3), 575–577. https://doi.org/10.1007/s10600-019-02748-x.

[3] Red Book of Uzbekistan. (2019). Endangered Plant Species. Tashkent: Chinor ENK. ISBN: 978-9943-03-097-8.

[4] George, E.F., Hall, M.A., De Klerk, G.J. (2008). Plant Propagation by Tissue Culture. Volume 1. Springer. https://doi.org/10.1007/978-1-4020-5005-3.

[5] Davies, P.J. (2010). Plant Hormones: Biosynthesis, Signal Transduction, Action! Springer. https://doi.org/10.1007/978-1-4020-2686-7.

[6] Karpov, A.A., Ivanova, N.N. (2020). Phytohormones: Functions and Applications. Moscow: Nauka. ISBN: 978-5-02-040123-4.

[7] Ozudogru, E.A., Kaya, E., Kirdok, E., et al. (2011). In vitro propagation of Thymus spathulifolius, an endemic species. Biologia Plantarum, 55(3), 423–430. https://doi.org/10.1007/s10535-011-0064-5.

[8] Gantait, S., Kundu, S. (2017). In vitro biotechnological approaches on Salvia sclarea. Plant Cell, Tissue and Organ Culture, 130 (2), 233–248. https://doi.org/10.1007/s11240-017-1234-8.

[9] Lattanzio, V., Cardinali, A., Ruta, C., et al. (2007). In vitro culture of Origanum vulgare. Plant Cell, Tissue and Organ Culture, 89(1), 55–64. https://doi.org/10.1007/s11240-007-9214-2.

[10] Santoro, M.V., Nievas, F.L., Zygadlo, J.A., et al. (2013). In vitro culture of Mentha piperita. Plant Cell, Tissue and Organ Culture, 113(3), 543–549. https://doi.org/10.1007/s11240-013 -0294-8.

[11] Thangavel, K., Maridass, M., Manoharan, C. (2013). In vitro regeneration of Pueraria phaseoloides. Plant Cell, Tissue and Organ Culture, 112(1), 113–119. https://doi.org/10.1007/s 11240-012-0220-6.

[12] Sultonova K.R., Kushiev Kh.Kh., Kodirov B.E. (2022). Root system formation and adaptation to ex vitro conditions in regenerant Lagochilus inebrians Bunge samples. E Conference Zone, 43–48. http://www.econferencezone.org/index.php/ecz/article/view/1491.

[13] Sultonova K., Kushiev Kh.Kh. Obtaining pathogen-free seedlings based on microclonal propagation of Lagochilus inebrians Bunge using in vitro methods. International Journal of Genetic Engineering, USA, 2022, 10(1): 10–15.

[14] Gollagunta, V., Adelberg, J.W., Rieck, J., Rajapakse, N. (2005). Sucrose in storage media and cultivar affects post-storage regrowth of in vitro Hosta propagules. Plant Cell, Tissue and Organ Culture, 80: 191–199.

[15] Gonçalves, S., Romano, A. (2013). In vitro culture of lavenders (Lavandula spp.) and the production of secondary metabolite. Biotechnology Advances, 31(2), 166–174. https://doi.org/10.1016/j.biotechadv.2012.09.006.

[16] Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

[17] Schneider, C.A., Rasband, W.S., Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671–675. https://doi.org/10.1038/nmeth.2089.

[18] Skoog, F., Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symposia of the Society for Experimental Biology, 11, 118–130. https://pubmed.ncbi.nlm.nih.gov/13486467/.

[19] Zhao, Y. (2010). Auxin biosynthesis and its role in plant development. Annual Review of Plant Biology, 61, 49–64. https://doi.org/10.1146/annurev-arplant-042809-112308.

[20] Hazarika, B.N. (2006). Morpho-physiological disorders in in vitro culture of plants. Scientia Horticulturae, 108(2), 105–120. https://doi.org/10.1016/j.scienta.2006.01.038.