Journal of Horticultural Research 2026, vol. 34(1): 11–22
DOI: 10.2478/johr-2026-0006

© The Author(s) 2026. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).

IN VITRO PROPAGATION AND FOLIAR MICROMORPHOLOGICAL STUDIES OF *DILLENIA INDICA* L.: AN UNDERUTILIZED FRUIT TREE

Amit SINGH, Srishti YADAV, Manoj K. RAI*
Department of Environmental Science,
Indira Gandhi National Tribal University, Amarkantak – 484887, M.P., India

Received: October 2025; Accepted: February 2026

ABSTRACT

The present study describes an improved, efficient method for *in vitro* propagation of the underutilized fruit tree, *Dillenia indica* L. Nodal segments obtained from a mature tree were used as initial explants for *in vitro* regeneration. Among the various cytokinin types (BAP, Kin, *m*T, and TDZ) and concentrations tested, MS medium containing 0.5 mg·L^{-1} 6-benzylaminopurine was optimal for shoot induction. Subculturing mother explants into fresh medium during subsequent passages significantly influenced shoot multiplication. A maximum of 5–7 shoots were regenerated after the third subculturing on MS medium containing 0.5 mg·L^{-1} 6-benzylaminopurine. Maximum rooting was achieved in MS medium containing 2 mg·L^{-1} indole-3-butyric acid. Approximately 80% of the plants survived acclimatization. A comparative study revealed significant changes in leaf micromorphology and architecture, including developmental and structural changes in venation patterns, trichomes, and stomata, in both *in vitro*-grown and fully acclimatized plants. This foliar micromorphological study elucidated the mechanism of adaptation of *in vitro*-regenerated plantlets during acclimatization. The current investigation could provide a foundation for the large-scale propagation and conservation of this medicinally important and underutilized fruit tree species.

Key words: acclimatization, *in vitro* regeneration, plant growth regulators, micromorphology

INTRODUCTION

Dillenia indica L., a member of the Dilleniaceae family, is an underutilized fruit tree grown in tropical and subtropical regions of many countries in Asia and Australia (Das et al. 2023; Saikumar et al. 2023). This tree grows primarily wild, and its fruit is edible for many animals, including birds and especially elephants, hence its popular name – elephant apple (Sharma et al. 2024). The fruit is rich in nutrients, with an excellent source of ascorbic acid, carbohydrates, carotenoids, fiber, minerals, protein, and vitamin C (Abdille et al. 2005; Saikia et al. 2023). Furthermore, it is used in the preparation of processed food products, like jam, jelly, and beverages, and locally for curries, pickles, and chutneys (Nayak et al. 2020; Saikumar et al. 2023). *D. indica* also exhibits notable medicinal attributes and is highly popular among indigenous and tribal populations in northeastern and central India. The leaves, bark, and fruit of this plant are used for gastrointestinal problems such as indigestion, colic, constipation, flatulence, anorexia, diarrhea, hyperacidity, stomach ache and jaundice, as well as respiratory ailments such as asthma, bronchitis, pneumonia, colds, influenza, mucus buildup, tonsillitis, and sore throat (Rai & Lalramnghinglova 2010; Saikia et al. 2023; Sharma et al. 2024). In recent years, this species has become well known in herbal and Ayurvedic medicine for its antidiabetic, antioxidant, antimicrobial, hepatoprotective, and anticancer properties (Kumar et al. 2010; Kamboj et al. 2019; Nayak et al. 2020). Several studies reported that this plant contains many active phytoconstituents such as alkaloids, terpenoids, flavonoids, glycosides, and polyphenols (Yazan & Armania 2014).

*Corresponding author
e-mail: mkraibhu@gmail.com

In India, this fruit tree is considered underutilized because it grows mostly wild, and is not typically cultivated commercially. Its fruit is primarily used by various tribal communities in Assam and northeastern India (Boruah et al. 2020; Das et al. 2023). Furthermore, there is no information on any commercial cultivars or commercial domestication (Das & Das 2003). Conventional breeding programs for improving many tree species are quite challenging due to prolonged breeding cycles, self-incompatibility, heterozygosity, limited genetic data, and the need to assess specific traits until the plant attains physiological maturity (Arora et al. 2022). Alternatively, several biotechnological approaches have substantial potential for improving many commercially important trees. However, biotechnological tree improvement programs require an efficient regeneration system (Peña & Séguin 2001). *D. indica* is conventionally propagated from seed or stem cuttings. However, seed propagation is not particularly suitable due to low seed germination, high heterozygosity, specific environmental requirements for germination, and mature seeds with underdeveloped embryos (Dasanayaka et al. 2022; Singh et al. 2026). Moreover, propagation of *D. indica* plants by stem cutting has sometimes been limited by diseases such as wilt or rot (Singh et al. 2026). Clonal propagation using cell, tissue, and organ culture techniques has become an effective and preferred approach, surpassing traditional propagation systems (Rai et al. 2010). Previously, *D. indica* received little research attention, with only a few papers published on its *in vitro* propagation (Abd El-Kadder & Hammad 2012; Taha et al. 2018). However, these studies were inefficient, and there is a need to improve existing protocols, particularly regarding shoot multiplication and rooting responses. A somatic embryogenesis system for *D. indica* using seed explant was recently developed (Singh et al. 2026). However, the low embryo induction rate and the presence of morphological abnormalities in the embryos remained an experimental challenge.

In vitro-grown plantlets develop under conditions of high humidity, low light, and a sugar-rich medium, leading to the development of plantlets with significant morphophysiological anomalies, such as thin cuticles, weak root systems, and non-functional or aberrant stomata. Such morphophysiological anomalies in *in vitro*-grown plantlets are not conducive to the acclimatization process (Pospíšilová et al. 1999; Hazarika 2006). Therefore, the transfer of plants from *in vitro* to the natural environment is a crucial phase, and careful monitoring of these morphophysiological alterations is essential.

The aim of this study is to develop an improved and efficient method for *in vitro* regeneration of *D. indica* using nodal explants from a mature tree. Furthermore, efforts were made to assess foliar micromorphological changes, i.e., developmental and structural changes in veins, trichomes, and stomata, in *D. indica* plantlets grown in tissue culture and compare them with plantlets acclimatized under *ex vitro* conditions.

MATERIALS AND METHODS

Young, freshly sprouted branches for culture initiation were collected from a 12–15-year-old *D. indica* tree growing on the campus of Indira Gandhi National Tribal University (Amarkantak, Madhya Pradesh, India) between April and August 2023. Nodal segments (2–3 cm long) were excised from the harvested shoots and used as explants for culture initiation. The explants were initially rinsed under running water for 20–25 min to remove any particulate matter. They were then submerged for 3–5 min in a 1% (v/v) solution of cetrimide (ICI India) and Tween 20, i.e., 2–3 drops in 100 ml of double-distilled water (DDW). During *D. indica* culture establishment, problems with leaching of phenolics and gums/latex were encountered. To minimize this, the explants were initially treated with NaCl (50 mg per 100 ml DDW) for 5–7 min and then with PVP (50 mg per 100 ml (w/v) polyvinylpyrrolidone) for 3–5 min. This was followed by a combination of chilled ascorbic acid and citric acid (100 mg per 100 ml and 50 mg per 100 ml DDW, respectively)

for 8–10 min. Surface sterilization was carried out under aseptic conditions in a laminar airflow hood in three steps. Initially, the explants were immersed in 0.1% (w/v) Bavistin (BASF, India) for 10 min, then in 2–3% (v/v) sodium hypochlorite solution for 4–5 min, and finally treated with 0.05% (w/v) $HgCl_2$ for 3–5 min. After each sterilization step, the explants were rinsed three times with DDW.

Culture media and conditions

Surface-sterilized nodal explants were cultured on MS (Murashige & Skoog 1962) basal media containing 3% (w/v) sucrose. All media were solidified using 0.8% (w/v) agar. Various plant growth regulators (PGRs), i.e., auxins and cytokinins, were added to the medium according to the experimental recommendations. Before adding agar to the medium, the pH was adjusted to 5.8 ± 0.02 with 1 N NaOH and 1 N HCl. The medium was then autoclaved at 121 °C and 103 kPa for 15 min. Cultures were maintained at 25 ± 2 °C, with a photoperiod of 16 : 8 h of light and dark, a photon flux of 50–60 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a relative humidity of 50–60%. All chemicals and PGRs used in this study were sourced from Hi-Media, India.

Effect of plant growth regulators on shoot induction and multiplication

For shoot induction, nodal explants were cultured in MS medium fortified with various concentrations of cytokinins (0.5, 1.0, 2.0, and 4.0 $mg \cdot L^{-1}$), including 6-benzylaminopurine (BAP), kinetin (Kin), metatopolin (*m*T), and thidiazuron (TDZ), alone or in combination with an optimized concentration of BAP (0.5 $mg \cdot L^{-1}$) and a low concentrations (0.1 or 0.3 $mg \cdot L^{-1}$) of indole-3-acetic acid (IAA) or α-naphthaleneacetic acid (NAA). Medium without PGRs served as a control. After 4 weeks of culture, the percentage response to shoot induction, average number of shoots, and shoot length were recorded. To enhance shoot multiplication, initial explants with shoots were repeatedly subcultured in optimized medium (MS + 0.5 $mg \cdot L^{-1}$ BAP) for 5 passages, 1 month apart. During subculturing, dried shoots and dead cells or tissue were carefully removed from the mother explants. Data on the average number and length of shoots were collected after 4 weeks.

***In vitro* rooting**

Shoots 4–5 cm long were individually excised from the multiplied shoots and transferred to MS basal medium supplemented with different auxins, namely indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), α-naphthaleneacetic acid (NAA), or 2-naphthoxyacetic acid (NOA) at concentrations of 1, 2, 3, and 4 $mg \cdot L^{-1}$. As a control, shoots were also placed on auxin-free medium. Data on percentage rooting, number of roots per shoot, and average root length were collected after 4 weeks after rooting.

Acclimatization of plantlets

Well-rooted, one-month-old plantlets were carefully removed from the medium, washed under running water, and placed in a pot with a mixture of autoclaved soil, sand, and commercial vermiculite (w/w 1 : 1 : 1). Initially, under laboratory conditions (25 ± 2 °C, 16-h photoperiod, illumination 40–50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PFD, and relative humidity 50–60%), the plantlets were covered with polythene bags to maintain humidity. After 1–2 months, the bags were removed, and well-acclimatized plantlets were transferred to earthen pots and moved to under field conditions. One month after transfer to the field, the survival of the acclimatized plantlets was recorded.

Foliar micromorphological analyses

Foliar micromorphological assessment was conducted on leaves obtained from both *in vitro*-derived shoots and well-acclimatized *D. indica* plants to compare the developmental and structural changes in leaf veins, trichomes, and stomata during adaptation from *in vitro* to field transfer. Leaves were collected from *in vitro*-derived plantlets (30 days after shoot regeneration) and well-acclimatized (30–45 days after field acclimatization) for foliar micromorphological assessments. The procedure for fixing leaf samples in FAA solution (formaldehyde, acetic acid, ethanol), dehydration, staining, and finally sectioning was carried out as described by Manokari and Shekhawat (2017). Photomicrographs were taken using a light microscope (Magnus Opto Systems, India). Stomata were observed under a light microscope at 10×40 magnification, while the venation pattern and trichomes in leaves were observed

at 10×10 magnification. Various foliar micromorphological parameters, i.e., stomatal index and density, trichome density, and vein architecture, were calculated using the equations adopted by Manokari and Shekhawat (2017). All micromorphological assessments were conducted thrice with 4–5 replicates.

Statistical analysis

The experiment was performed in a completely randomized design (CRD) for a single factor. Each experiment was conducted three times with fifteen replicates per repetition. One replicate represents one nodal explant cultured in one test tube. Results are expressed as the mean ± SE of three repeated experiments. Data were statistically analyzed using SPSS vs 20. One-way analysis of variance (ANOVA) was conducted to assess differences between means, and significant differences between means were assessed using the least significant difference (LSD) and Duncan's multiple range test (DMRT) at $P \leq 0.05$.

RESULTS AND DISCUSSION

Culture establishment

In the current study, establishing axenic *D. indica* cultures from explants taken from mature trees proved difficult due to culture contamination, season-dependent responses, and leaching of phenolics and gums from the cut ends of the explants. The lateral branches from which the nodal explants were obtained displayed hairy and thick shoots that harbored numerous fungal and bacterial contaminants. Thus, a systemic fungicide (Bavistin) and surface-sterilizing agents (sodium hypochlorite, mercuric chloride) were used to establish a microbial-free culture of *D. indica*. The rate of culture contamination and the performance of nodal segments in shoot induction varied with the season. After the spring season, in April and May, new, healthy sprouts appeared on existing branches. Explants taken from these sprouts performed well in culture establishment, with a low rate of culture contamination (5–10%) and better shoot induction. During the rainy season (June–August), bacterial and fungal contamination markedly increased (70–80%), which caused difficulties in culture establishment. During the winter season, microbial contamination was relatively low, but the explants performed poorly, likely due to their maturity and dormancy. The effect of seasonal changes on explant performance in the present study may be correlated with their physiological state, which is influenced by the annual growth cycle of trees, i.e., the end of the dormancy period after winter and the beginning of the active growth period in spring (Chhajer & Kalia 2017; Asthana et al. 2024; Yadav et al. 2025). After standardizing the explant collection seasons in April and May (2023), all further shoot induction and multiplication experiments were performed in the subsequent two years (2024–2025) on explants collected only in April and May.

During culture establishment, the cut ends of nodal explants produced high levels of phenolic exudates, leading to their browning. Excessive phenolic exudation from cut ends and explant browning are two major obstacles to tissue culture in woody plant species (Arora et al. 2022). The synthesis of phenolic compounds in woody plants is determined by genetic and environmental factors, especially as a protective response to biotic and abiotic stresses (Lux-Endrich et al. 2000; Liu et al. 2024). However, compared to herbaceous and annual plants, perennial woody species synthesize phenolic compounds in excess because they are exposed to multiple stresses, such as heat, drought, and cold throughout the year (Vibha et al. 2014). Explant browning involves the enzymatic oxidation of polyphenols released from the cut ends of the explants, leading to the formation of quinones, which cause tissue browning and inhibit culture growth (Liu et al. 2024). Phenolic exudation can be reduced by rinsing the explants with NaCl (50 mg per 100 ml DDW), PVP (50 mg per 100 ml), and then with a cooled antioxidant solution containing ascorbic acid and citric acid (100 mg per 100 ml and 50 mg per 100 ml DDW, respectively). Antioxidants and

PVP have been shown to significantly control phenolic exudation in many woody plant species (Pandey et al. 2006; Rai et al. 2010; Phulwaria et al. 2012; Rathore et al. 2014; Sharma et al. 2017; Ekka et al. 2020; Patel et al. 2020; Shekhawat et al. 2021). The beneficial effects of antioxidants may be attributed to their ability to scavenge reactive oxygen species generated by wounded explants during culture establishment, while PVP acts as a phenol adsorbent, reducing phenol leaching and slowing the progression of tissue necrosis (Krishna et al. 2008; Permadi et al. 2024). NaCl has also been reported to control tissue browning in *Paeonia lactiflora* (Cai et al. 2020). NaCl has been suggested for browning control because it inhibits polyphenol oxidase (PPO) activity, which is primarily involved in phenolic synthesis in plants.

Shoot induction and multiplication

The response of nodal explants to shoot induction in *D. indica* was strongly dependent on the type of cytokinin and its concentration (Table 1). Nodal explants cultured on MS media without cytokinin also exhibited shoot induction, with a response rate of 82.2 ± 4.4%. It appears that the nutrients of the basal MS medium stimulated preexisting meristems, thereby causing the proliferation of 1–2 shoots from nodal explants on medium without cytokinin. The performance of nodal explants, measured by the percentage response to shoot induction, the number of shoots per explant, and average shoot length, varied depending on the type of cytokinin. The most effective cytokinins for shoot induction were BAP > Kin > *m*T > TDZ (Table 1). Selecting the suitable cytokinin and its proper concentration is essential for establishing a regeneration protocol for any plant species. The results of this study clearly showed that BAP performed better than other cytokinins in shoot induction in *D. indica*. Due to its ability to stimulate cell division and meristem activation, BAP is considered the most common and highly rated cytokinin, widely used for axillary bud proliferation and multiple shoot induction (Bhojwani & Dantu 2013; Rademacher 2015).

Table 1. Effect of cytokinins on shoot induction from *Dillenia indica* nodal explants

Cytokinin	Concentrations ($mg \cdot L^{-1}$)	Response to shoot induction (%)	Average shoot number	Average shoot length (cm)
Control	0.0	82.2±4.4abc	1.4±0.1cdef	1.50±0.08e
BAP	0.5	93.3±3.8a	2.11±0.15a	2.07±0.11a
	1.0	88.8±2.2abc	1.73±0.10b	1.96±0.11abc
	2.0	84.4±5.8abc	1.41±0.10cdef	1.90±0.09abc
	4.0	75.5±5.8cd	1.32±0.09def	1.85±0.06abc
Kin	0.5	91.1±5.8ab	1.73±0.15b	1.97±0.16ab
	1.0	88.8±2.2abc	1.66±0.04bc	1.62±0.21cd
	2.0	84.4±5.8abc	1.58±0.07bcd	1.44±0.11de
	4.0	77.7±5.8bcd	1.29±0.08def	1.34±0.05de
*m*T	0.5	88.8±2.2abc	1.53±0.08bcde	1.85±0.07abc
	1.0	84.4±2.2abc	1.43±0.16bcdef	1.63±0.09bcd
	2.0	82.2±2.2abc	1.31±0.09def	1.46±0.11de
	4.0	66.6±3.8de	1.15±0.10f	1.26±0.08e
TDZ	0.5	55.5±2.2ef	1.19±0.03ef	1.09±0.04ef
	1.0	51.1±2.2fg	1.08±0.04ef	0.85±0.13f
	2.0	51.1±2.2fg	0.87±0.06g	0.59±0.01g
	4.0	42.2±2.2g	0.72±0.03g	0.48±0.01g
LSD ($P \leq 0.05$)		11.69	0.28	0.3
ANOVA		$F_{16,34} = 13.58$	$F_{16,34} = 9.363$	$F_{16,34} = 16.186$

Medium: 0.8% agar-solidified medium + 3% sucrose; Mean values followed by different letters under different treatments within a column are significantly different from each other at $P \leq 0.05$ according to Duncan's multiple range test

Regardless of the cytokinins and their concentration, shoot initiation from nodal explants was observed within 10–14 days of culture (Fig. 1A). Of all the cytokinin concentrations tested, 0.5 mg·L^{-1} BAP was found the most effective in shoot induction with a maximum response of 93.3 ± 3.8% and 2.11 ± 0.15 shoots per explant (Table 1, Fig. 1B). The interaction of 0.5 mg·L^{-1} of BAP with low concentrations (0.1 or 0.3 mg·L^{-1}) of IAA or NAA auxins was also tested for shoot induction. However, the interactive effect of BAP with auxins was found to be suppressive for both the percentage response to shoot induction and the average number of shoots (Table 2). The suitability of lower concentrations of BAP for shoot induction may be due to the presence of sufficient endogenous cytokinin in the explants. Supplying higher amounts of exogenous cytokinin, alone or in combination with auxin, may lead to excessive accumulation of the hormone in plant tissues and thus inhibit shoot formation (Bhojwani & Dantu 2013). Similar to our observations, BAP alone and in lower concentrations was also found to induce shoots in many trees, like *Psidium guajava* (Rai et al. 2009), *Azadirachta indica* (Arora et al. 2010), *Garcinia indica* (Malik et al. 2005), *Salix nigra* (Lyyra et al. 2006), *Betula lenta* (Rathwell et al. 2016), and *Aflatunia ulmifolia* (Kirillov et al. 2024).

Table 2. Effect of auxin–cytokinin interaction on shoot induction from *Dillenia indica* nodal explants

Plant growth regulator concentration (mg·L^{-1})			Response to shoot induction (%)	Average number of shoots per explant	Average shoot length (cm)
BAP	IAA	NAA			
0.5			93.3±3.8a	2.11±0.15a	2.07±0.11a
0.5	0.1		82.1±3.0b	1.51±0.19b	1.48±0.03b
0.5	0.3		75.5±4.8b	1.30±0.12b	1.31±0.07b
0.5		0.1	78.8±2.2b	1.27±0.09b	1.45±0.09b
0.5		0.3	72.2±2.9b	1.18±0.03b	1.25±0.03b
LSD ($P \leq 0.05$)			11.01	0.41	0.23
ANOVA			$F_{4,10} = 3.37$	$F_{4,10} = 7.87$	$F_{4,10} = 18.90$

Medium: 0.8% agar-solidified medium + 3% sucrose; Mean values followed by different letters under different treatments within a column are significantly different from each other at $P \leq 0.05$ according to Duncan's multiple range test



Figure 1. Shoot induction and multiplication from nodal segments of *Dillenia indica*: bud breaking from a nodal explant on MS medium with BAP (0.5 mg·L^{-1}) within 2 weeks (A); induction of 3–4 shoots from a nodal explant on MS medium with BAP (0.5 mg·L^{-1}) within 4 weeks (B); shoot multiplication on MS medium with BAP (0.5 mg·L^{-1}) after the third monthly subculture of the mother explant (C); *in vitro* rooting on MS medium with IBA (2.0 mg·L^{-1}) (D & E); 2–3-month-old, well-acclimatized *D. indica* plant (F)
Scale bar: A & C – 1.0 cm, B & E – 1.5 cm, D – 2.0 cm, F – 5.0 cm

For shoot multiplication, the mother nodal segments, along with the induced shoots, were transferred to MS medium containing an optimized concentration of BAP (0.5 mg·L^{-1}) and repeated for five passages at 1-month intervals. Monthly subculture of the mother explants to fresh medium significantly affected shoot multiplication. The number of shoots increased until the third transfer and then decreased significantly (Table 3). A maximum of 5–7 shoots with a mean shoot number of 5.62 ± 0.1 were regenerated after the third subculture on medium containing 0.5 mg·L^{-1} BAP (Fig. 1C). Regular subculture of the mother explants not only provides fresh nutrients, thus avoiding nutrient deficiencies, but also leads to shoots rejuvenation, resulting in enhanced shoot multiplication (Martínez et al. 2017). However, the decrease in shoot multiplication potential after the third subculture may be due to residual hormonal effects and the accumulation of inhibitory substances resulting from prolonged culture (Vujović et al. 2012).

Table 3. Effect of subculture on shoot multiplication in *Dillenia indica*

Number of transfers	Average number of shoots
1	3.5±0.3c
2	4.2±0.2b
3	5.6±0.1a
4	4.2±0.08b
5	3.8±0.1bc
LSD ($P \leq 0.05$)	0.56
ANOVA	$F_{4,10} = 20.93$

Medium: 0.8% agar-solidified MS medium + BAP (0.5 mg·L^{-1}) + 3% sucrose; Mean values followed by different letters under different treatments within a column are significantly different from each other at $P \leq 0.05$ according to Duncan's multiple range test

Rooting and acclimatization of plants *in vitro*

In the present study, four auxins, namely IBA, IAA, NAA, and NOA, were tested separately for *in vitro* rooting in *D. indica* shoots. However, IAA, NAA, and NOA completely failed to induce root formation in shoots. *In vitro* rooting was observed only on MS medium containing 1–3 mg·L^{-1} IBA. No rooting was obtained on medium lacking IBA (control) or at a higher concentration of IBA (4 mg·L^{-1}). The best rooting performance was recorded on MS medium containing 2 mg·L^{-1} IBA (Table 4). Approximately 86% of shoots rooted in this medium, with a maximum number of roots per shoot (11.6 ± 0.8) and an average root length of 8.5 cm (Table 4; Fig. 1D–E). Root initiation was evident within 2 weeks of IBA treatment. The success of micropropagation of any plant largely depends on effective and reasonable shoot rooting responses, which are necessary for successful field acclimatization. *In vitro* rooting is quite difficult in woody plant species and often depends on several factors, such as auxin types and concentrations, duration of auxin treatment, medium strength, and shoot developmental stage of (De Klerk et al. 1999; Giri et al. 2004; Ragonezi et al. 2010; Arora et al. 2022). Of several auxins used for rooting, IBA has been reported to be the best auxin for both *in vitro* rooting and rooting of cuttings or in other propagation methods, like aeroponics or hydroponics (Bhojwani & Dantu 2013; Mehandru et al. 2014; Scaltrito et al. 2024; Zalt et al. 2024; Gabryszewska et al. 2025). Better rooting of shoots treated with IBA is mainly due to its greater stability and easier uptake by plant tissues (Martínez et al. 2017). In tissue culture of woody plants, IBA is widely used to enhance rooting, even in difficult-to-root species (Phulwaria et al. 2012; Rathore et al. 2014; Patel et al. 2020; Shekhawat et al. 2021; Asthana et al. 2024). Approximately 80% of the plantlets survived after acclimatization (Fig. 1F).

Table 4. Effect of indole-3-butyric acid (IBA) on the rooting of *Dillenia indica* shoots *in vitro*

IBA concentration (mg·L^{-1})	Response to shoot induction (%)	Number of roots per shoot	Average root length (cm)
0	0.0±0.0d	0.0±0.0c	0.0±0.0c
1	63.3±5.1b	5.6±0.8b	3.3±0.5b
2	86.0±3.3a	11.6±0.8a	8.5±0.6a
3	37.4±9.5c	4.0±0.6b	3.5±0.3b
4	0.0±0.0d	0.0±0.0c	0.0±0.0c
LSD ($P \leq 0.05$)	15.89	1.93	1.29
ANOVA	$F_{4,10} = 56.90$	$F_{4,10} = 61.67$	$F_{4,10} = 72.158$

Medium: 0.8% agar-solidified medium + 3% sucrose; Mean values followed by different letters under different treatments within a column are significantly different from each other at $P \leq 0.05$ according to Duncan's multiple range test

Foliar micromorphological studies

In vitro-grown plantlets usually grow in high humidity, low light, and sugar-rich media, leading to abnormal morphophysiological traits (Bhojwani & Dantu 2013). Understanding the foliar micromorphological features of *in vitro*-grown plants, especially anatomical anomalies and their structural adjustments during acclimatization, is vital for developing a successful protocol for plant regeneration. Providing valuable information on structural changes will enable tissue culture-grown plantlets to survive in the field and natural environments (Pospíšilová et al. 1999; Hazarika 2006; Sharma et al. 2023).

In the present study, a comparative assessment of foliar micromorphological features and their architecture, i.e., developmental and structural changes in venation patterns, trichomes, and stomata, was conducted in both *in vitro*-grown and fully acclimatized *D. indica* plants, and some notable changes were observed in microscopic analyses. The venation pattern was less pronounced in *in vitro* leaves, with a lower density and incomplete vein network (Fig. 2A). In contrast, acclimatized plants showed a well-developed venation pattern with increased vein density (Fig. 2B). A significant quantitative change in the vein islets (VI) and veinlet terminations (VT) was also observed in both *in vitro* and acclimatized plants. In *in vitro* plants, the number of VI was approximately 6.00 VI per mm^2, representing a nearly two-fold increase (12.25 VI per mm^2) after 4 weeks under field conditions. Similarly, the number of VT also increased from *in vitro* plantlets (2.95 VT per mm^2) to acclimatized plants (4.27 VT per mm^2). The well-developed venation pattern and increased number of vein islets and veinlet terminations in acclimatized plants support environmental adaptation by developing vascular tissues, maturing leaves, and ensuring proper mineral and nutrient translocation (Shekhawat & Manokari 2017; Manokari et al. 2022). Similarly, trichome density was noticeably lower in leaves of *in vitro*-derived plantlets compared to acclimatized plants (Fig. 2C–D). Moreover, trichomes were underdeveloped and smaller in leaves of *in vitro* plants, which became fully developed, larger, and thicker after acclimatization of the plantlets to field conditions (Fig. 2C–D). The marked increase in trichome density during hardening reflects an adaptive feature that strengthens plant natural defense system under both biotic and abiotic stress (Bandyopadhyay et al. 2004; Shekhawat & Manokari 2017; Grzelak et al. 2024).

Stomatal structure and distribution also differed between leaves of *in vitro* and acclimatized plants. Leaves of *in vitro*-derived plantlets displayed stomata with large guard cells that were always open, regardless of the time and culture conditions, indicating their nonfunctional status (Fig. 2E). In contrast, plants acclimatized to field conditions developed well-structured and functional stomata, with apertures that were either partially open or closed in response to surrounding environmental factors (Fig. 2F). Leaves of *in vitro*-derived plantlets possessed stomata in greater numbers compared with acclimatized plants (Fig. 2E–F). Quantitative analysis of the stomatal index (SI) also showed a higher SI of 30.5 ± 1.75 in *in vitro*-grown plants, which decreased to 23.1 ± 0.27 in plants transferred to the field. *In vitro*, plants grow under artificial light and high relative humidity, requiring sugars as an energy source and growth regulators, which interfere with the natural development of stomata and photosynthetic apparatus (Pospíšilová et al. 1999; Hazarika 2006; Grzelak et al. 2024). Thus, *in vitro*-grown plants produced a large number of open, abnormal, and nonfunctional stomata with a high stomatal index, which correlated with reduced acclimation success due to excessive water loss. During acclimatization, plants underwent a number of morphophysiological adaptations, including the development of normal stomata with reduced stomatal density, well-developed venation patterns with an increased vein islets, veinlet terminations, and trichome density, etc. Similar to this study, several others also reported that these foliar micromorphological changes facilitated the survival of *in vitro*-grown plants during the acclimatization process (Rathore et al. 2013; Lodha et al. 2015; Manokari & Shekhawat 2017; Sharma et al. 2017; Shekhawat & Manokari 2017; Choudhary et al. 2020; Manokari et al. 2020; Chouhan et al. 2025).

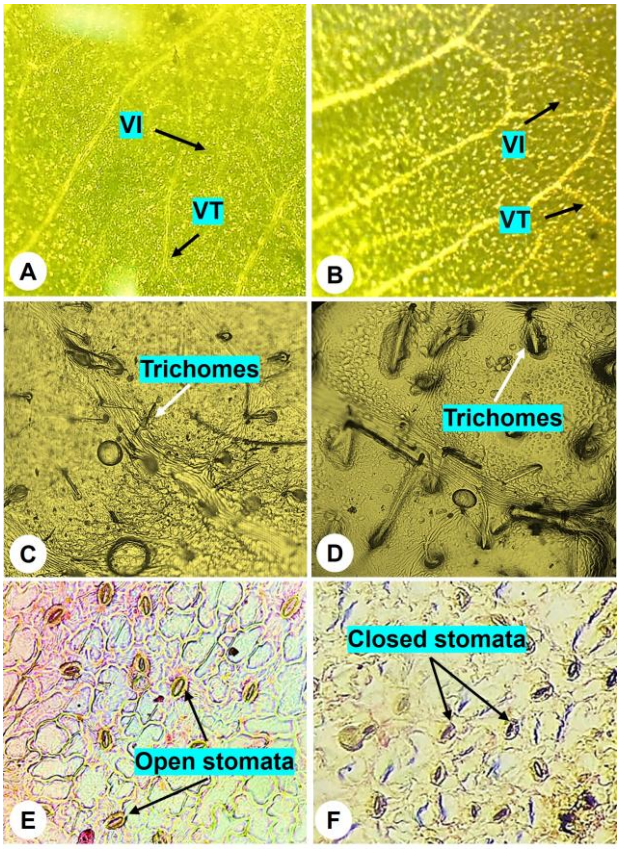


Figure 2. Foliar micromorphological studies of *D. indica* plants grown *in vitro* and transferred to the field: venation pattern in leaves of *in vitro*-grown shoot (A) and the hardened plant (B), VI – vein islet, VT – veinlet termination, magnification 10×10; trichomes in leaves of *vitro*-grown shoot (C) and the hardened plant (D), magnification 10×10; stomata of *in vitro*-grown shoot (E) and the hardened plant (F), magnification 10×40

CONCLUSION

An improved, more efficient *in vitro* regeneration protocol for *Dillenia indica* was developed using nodal explants taken from mature trees. However, culture establishment was challenging due to excessive leaching of phenolics from the cut ends, culture contamination, and season-dependent responses. The best time to collect initial explants was April–March. Despite these problems, the culture was successfully established. Shoot induction was obtained on MS medium containing 0.5 mg·L^{-1} 6-benzylaminopurine. Further increases in shoot production occurred after subsequent transfer of the mother explants to fresh media. The protocol could serve as a basis for large-scale propagation and conservation of this medicinally important, underutilized fruit tree species. The findings of foliar micromorphological studies could help understand the adaptation and improved survival of tissue cultured plants under field conditions.

Author contribution statement

AS – A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article

SY – B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article

MR – A – Research concept and design, E – Critical revision of the article, F – Final approval of the article

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Abd El-Kadder E.M., Hammad H.H. 2012. In vitro propagation of *Dillenia indica*. Australian Journal of Basic and Applied Sciences 6(7): 452–457.

Abdille M.H., Singh R.P., Jayaprakasha G.K., Jena B.S. 2005. Antioxidant activity of the extracts from *Dillenia indica* fruits. Food Chemistry 90(4): 891–896. DOI: 10.1016/j.foodchem.2004.09.002.

Arora K., Rai M.K., Sharma A.K. 2022. Tissue culture mediated biotechnological interventions in medicinal trees: recent progress. Plant Cell, Tissue and Organ Culture 150(2): 267–287. DOI: 10.1007/s11240-022-02298-1.

Arora K., Sharma M., Srivastava J., Ranade S.A., Sharma A.K. 2010. Rapid in vitro cloning of a 40-year-old tree of *Azadirachta indica* A.Juss. (neem) employing nodal stem segments. Agroforestry Systems 78(1): 53–63. DOI: 10.1007/s10457-009-9230-1.

Asthana P., Rai M.K., Jaiswal U. 2024. Factors affecting the micropropagation of *Sapindus trifoliatus* L. from nodal explants of mature tree. Current Biotechnology 13(1): 58–67. DOI: 10.2174/0122115501289941240109061526.

Bandyopadhyay T., Gangopadhyay G., Poddar R., Mukherjee K.K. 2004. Trichomes: their diversity, distribution and density in acclimatization of teak (*Tectona grandis* L.) plants grown *in vitro*. Plant Cell, Tissue and Organ Culture 78(2): 113–121. DOI: 10.1023/b:ticu.0000022534.03276.c5.

Bhojwani S.S., Dantu P.K. 2013. Plant tissue culture: an introductory text. Springer, India, 309 p. DOI: 10.1007/978-81-322-1026-9.

Boruah R., Saikia S., Islam S., Purkayastha M.D., Borah A. 2020. *Dillenia indica*: an underutilized fruit with gamut of food and industrial prospectives. Medicinal Plants 12(2): 214–219. DOI: 10.5958/0975-6892.2020.00028.3.

Cai X., Wei H., Liu C., Ren X., Thi L.T., Jeong B.R. 2020. Synergistic effect of NaCl pretreatment and PVP on browning suppression and callus induction from petal explants of *Paeonia lactiflora* Pall. 'Festival Maxima'. Plants 9(3); 346; 13 p. DOI: 10.3390/plants9030346.

Chhajer S., Kalia R.K. 2017. Seasonal and micro-environmental factors controlling clonal propagation of mature trees of marwar teak [*Tecomella undulata* (Sm.) Seem]. Acta Physiologiae Plantarum 39(2); 60; 15 p. DOI: 10.1007/s11738-017-2364-2.

Choudhary D., Rai M.K., Shekhawat N.S., Kataria V. 2020. In vitro propagation of *Farsetia macrantha* Blatt. & Hallb.: an endemic and threatened plant of Indian Thar Desert. Plant Cell, Tissue and Organ Culture 142(3): 519–526. DOI: 10.1007/s11240-020-01876-5.

Chouhan R., Shekhawat N.S., Patel A.K., Ram K., Choudhary A., Ambawat S., Choudhary S.K. 2025. *Meta*-topolin mediated enhanced micropropagation, foliar-micromorphological evaluation and genetic homogeneity validation in African pumpkin (*Momordica balsamina* L.). South African Journal of Botany 180: 286–296. DOI: 10.1016/j.sajb.2025.03.005.

Das M., Choudhury M., Sarma B.P. 2023. *Dillenia indica* L. In: Belwal T., Bhatt I.D., Devkota H.P. (Eds.), Himalayan fruits and berries. Academic Press, India, pp. 111–122. DOI: 10.1016/b978-0-323-85591-4.00021-0.

Das B.C., Das S.N. 2003. Cultivation of minor fruits. Kalyani Publishers, India.

Dasanayaka B.I., Jinadasa R.N., Jayasuriya K.M.G.G., Phartyal S.S. 2022. Seed ecophysiology of elephant apple (*Dillenia indica*) – an important tree species of the Indomalayan realm. Ecological Research 37(4): 532–543. DOI: 10.1111/1440-1703.12312.

De Klerk G.J., Van der Krieken W., De Jong J.C. 1999. The formation of adventitious roots: new concepts, new possibilities. In Vitro Cellular and Developmental Biology – Plant 35(3): 189–199. DOI: 10.1007/s11627-999-0076-z.

Ekka G., Jadhav S.K., Quraishi A. 2020. Effect of exogenous additives on oxidative stress and defense system of a tree: *Zanthoxylum armatum* DC. under in vitro conditions. Plant Cell, Tissue and Organ Culture 140(3): 671–676. DOI: 10.1007/s11240-019-01759-4.

Gabryszewska E.A., Norwa K., Norwa P., Majos D. 2025. Effect of medium composition and culture conditions on the *in vitro* multiplication, rooting and acclimatization of *Helleborus* 'Molly's White' and 'Anna's Red'. Journal of Horticultural Research 33(2): 41–58. DOI: 10.2478/johr-2025-0016.

Giri C.C., Shyamkumar B., Anjaneyulu C. 2004. Progress in tissue culture, genetic transformation and applications of biotechnology to trees: an overview. Trees 18(2): 115–135. DOI: 10.1007/s00468-003-0287-6.

Grzelak M., Pacholczak A., Nowakowska K. 2024. Challenges and insights in the acclimatization step of micropropagated woody plants. Plant Cell, Tissue and Organ Culture 159(3); 72; 20 p. DOI: 10.1007/s11240-024-02923-1.

Hazarika B.N. 2006. Morpho-physiological disorders in in vitro culture of plants. Scientia Horticulturae 108(2): 105–120. DOI: 10.1016/j.scienta.2006.01.038.

Kamboj P., Talukdar N.C., Banerjee S.K. 2019. Therapeutic benefit of *Dillenia indica* in diabetes and its associated complications. Journal of Diabetes Research 2019; 4632491; 7 p. DOI: 10.1155/2019/4632491.

Kirillov V., Pathak A., Patel S.R., Serafimovich M., Daulenova M., Gorbachenkov M. et al. 2024. An efficient micropropagation protocol for an endangered tree species *Aflatunia ulmifolia* (Franch.) Vassilcz. In Vitro Cellular and Developmental Biology – Plant 60(1): 28–38. DOI: 10.1007/s11627-023-10392-y.

Krishna H., Sairam R.K., Singh S.K., Patel V.B., Sharma R.R., Grover M. et al. 2008 Mango explants browning: Effect of ontogenic age, mycorrhization and pre-treatments. Scientia Horticulturae 118(2): 132–138. DOI: 10.1016/j.scienta.2008.05.040.

Kumar D., Mallick S., Vedasiromoni J.R., Pal B.C. 2010. Anti-leukemic activity of *Dillenia indica* L. fruit extract and quantification of betulinic acid by HPLC. Phytomedicine 17(6): 431–435. DOI: 10.1016/j.phymed.2009.07.010.

Liu C., Fan H., Zhang J., Wu J., Zhou M., Cao F. et al. 2024. Combating browning: mechanisms and management strategies in *in vitro* culture of economic woody plants. Forestry Research 4; e032; 11 p. DOI: 10.48130/forres-0024-0026.

Lodha D., Patel A.K., Shekhawat N.S. 2015. A high-frequency *in vitro* multiplication, micromorphological studies and *ex vitro* rooting of *Cadaba fruticosa* (L.) Druce (Bahuguni): a multipurpose endangered medicinal shrub. Physiology and Molecular Biology of Plants 21(3): 407–415. DOI: 10.1007/s12298-015-0310-6.

Lux-Endrich A., Treutter D., Feucht W. 2000. Influence of nutrients and carbohydrate supply on the phenol composition of apple shoot cultures. Plant Cell, Tissue and Organ Culture 60(1): 15–21. DOI: 10.1023/a:1006406527242.

Lyyra S., Lima A., Merkle S. 2006. *In vitro* regeneration of *Salix nigra* from adventitious shoots. Tree Physiology 26(7): 969–975. DOI: 10.1093/treephys/26.7.969.

Malik S.K., Chaudhury R., Kalia R.K. 2005. Rapid in vitro multiplication and conservation of *Garcinia indica*: a tropical medicinal tree species. Scientia Horticulturae 106(4): 539–553. DOI: 10.1016/j.scienta.2005.05.002.

Manokari M., Mathiyazhagan C.R., Selvam P., Phulwaria M., Shekhawat M.S. 2020. Foliar micro-morphology: a promising tool to improve survival percentage of tissue culture raised plantlets with special reference to *in vitro* propagation of *Vitex negundo* L. Vegetos 33(3): 504–515. DOI: 10.1007/s42535-020-00134-5.

Manokari M., Priyadharshini S., Shekhawat M.S. 2022. Repairing mechanism of foliar micro-morphological anomalies during acclimatization and field transfer of in vitro raised plantlets of *Aerva lanata* (L.) Juss. ex Schult.: a medicinally important plant. Vegetos 35(2): 520–526. DOI: 10.1007/s42535-021-00317-8.

Manokari M., Shekhawat M.S. 2017. Foliar micromorphology of *in vitro*-cultured shoots and field-grown plants of *Passiflora foetida*. Horticultural Plant Journal 3(1): 34–40. DOI: 10.1016/j.hpj.2017.01.009.

Martínez M.T., Corredoira E., Vieitez A.M., Cernadas M.J., Montenegro R., Ballester et al. 2017. Micropropagation of mature *Quercus ilex* L. trees by axillary budding. Plant Cell, Tissue and Organ Culture 131(3): 499–512. DOI: 10.1007/s11240-017-1300-x.

Mehandru P., Shekhawat N.S., Rai M.K., Kataria V., Gehlot H.S. 2014. Evaluation of aeroponics for clonal propagation of *Caralluma edulis*, *Leptadenia reticulata* and *Tylophora indica* – three threatened medicinal Asclepiads. Physiology and Molecular Biology of Plants 20(3): 365–373. DOI: 10.1007/s12298-014-0240-8.

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15(3): 473–497. DOI: 10.1111/j.1399-3054.1962.tb08052.x.

Nayak S., Bhat M.P., Udayashankar A.C., Lakshmeesha T.R., Geetha N., Jogaiah S. 2020. Biosynthesis and characterization of *Dillenia indica*-mediated silver nanoparticles and their biological activity. Applied Organometallic Chemistry 34(4); e5567; 9 p. DOI: 10.1002/aoc.5567.

Pandey S., Singh M., Jaiswal U., Jaiswal V.S. 2006. Shoot initiation and multiplication from a mature tree of *Terminalia arjuna* Roxb. In Vitro Cellular and Developmental Biology – Plant 42(5): 389–393. DOI: 10.1079/ivp2006790.

Patel A.K., Lodha D., Shekhawat N.S. 2020. An improved micropropagation protocol for the *ex situ* conservation of *Mitragyna parvifolia* (Roxb.) Korth. (Rubiaceae): an endangered tree of pharmaceutical importance. In Vitro Cellular and Developmental Biology – Plant 56(6): 817–826. DOI: 10.1007/s11627-020-10089-6.

Peña L., Séguin A. 2001. Recent advances in the genetic transformation of trees. Trends in Biotechnology 19(12): 500–506. DOI: 10.1016/s0167-7799(01)01815-7.

Permadi N., Akbari S.I., Prismantoro D., Indriyani N.N., Nurzaman M., Alhasnawi A.N. et al. 2024. Traditional and next-generation methods for browning control in plant tissue culture: Current insights and future directions. Current Plant Biology 38; 100339; 15 p. DOI: 10.1016/j.cpb.2024.100339.

Phulwaria M., Rai M.K., Harish, Gupta A.K., Ram K., Shekhawat N.S. 2012. An improved micropropagation of *Terminalia bellirica* from nodal explants of mature tree. Acta Physiologiae Plantarum 34(1): 299–305. DOI: 10.1007/s11738-011-0828-3.

Pospíšilová J., Tichá I., Kadleček P., Haisel D., Plzáková Š. 1999. Acclimatization of micropropagated plants to *ex vitro* conditions. Biologia Plantarum 42(4): 481–497. DOI: 10.1023/a:1002688208758.

Rademacher W. 2015. Plant growth regulators: backgrounds and uses in plant production. Journal of Plant Growth Regulation 34(4): 845–872. DOI: 10.1007/s00344-015-9541-6.

Ragonezi C., Klimaszewska K., Castro M.R., Lima M., de Oliveira P., Zavattieri M.A. 2010. Adventitious rooting of conifers: influence of physical and chemical factors. Trees – Structure and Function 24(6): 975–992. DOI: 10.1007/s00468-010-0488-8.

Rai M.K., Asthana P., Jaiswal V.S., Jaiswal U. 2010. Biotechnological advances in guava (*Psidium guajava* L.): recent developments and prospects for further research. Trees – Structure and Function 24(1): 1–12. DOI: 10.1007/s00468-009-0384-2.

Rai M.K., Jaiswal V.S., Jaiswal U. 2009. Shoot multiplication and plant regeneration of guava (*Psidium guajava* L.) from nodal explants of *in vitro* raised plantlets. Journal of Fruit and Ornamental Plant Research 17(1): 29–38.

Rai P.K., Lalramnghinglova H. 2010. Ethnomedicinal plant resources of Mizoram, India: implication of traditional knowledge in health care system. Ethnobotany Leaflets 14(3): 274–305.

Rathore J.S., Rai M.K., Phulwaria M., Shekhawat N.S. 2014. A liquid culture system for improved micropropagation of mature *Acacia nilotica* (L.) Del. ssp. *indica* and ex vitro rooting. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 84(1): 193–200. DOI: 10.1007/s40011-013-0204-8.

Rathore N.S., Rathore N., Shekhawat N.S. 2013. In vitro propagation and micromorphological studies of *Cleome gynandra*: a C_4 model plant closely related to *Arabidopsis thaliana*. Acta Physiologiae Plantarum 35(9): 2691–2698. DOI: 10.1007/s11738-013-1301-2.

Rathwell R., Shukla M.R., Jones A.M.P., Saxena P.K. 2016. In vitro propagation of cherry birch (*Betula lenta* L.). Canadian Journal of Plant Science 96(4): 571–578. DOI: 10.1139/cjps-2015-0331.

Saikia D., Kesavan R., Inbaraj B.S., Dikkala P.K., Nayak P.K., Sridhar K. 2023. Bioactive compounds and health-promoting properties of elephant apple (*Dillenia indica* L.): a comprehensive review. Foods 12(16); 2993; 25 p. DOI: 10.3390/foods12162993.

Saikumar A., Nickhil C., Badwaik L.S. 2023. Physicochemical characterization of elephant apple (*Dillenia indica* L.) fruit and its mass and volume modeling using computer vision. Scientia Horticulturae 314; 111947; 8 p. DOI: 10.1016/j.scienta.2023.111947.

Scaltrito E., Cristiano G., Sdao A.E., Gruda N.S., Loconsole D., De Lucia B. 2024. Influence of water spraying intervals and indole-3-butyric acid concentrations on *Salvia* rooted cuttings quality in a closed aeroponics system. Scientia Horticulturae 337; 113452; 12 p. DOI: 10.1016/j.scienta.2024.113452.

Sharma B.P., Sureshrao G.A., Kshirsagar J.J., Kumar S. 2024. Unveiling the potential of *Dillenia indica* L.: A comprehensive review. In: Sharma B.P., Suple S.D., Mishra S., Rath S.K. (Eds.), Plants and secondary metabolites, vol. 3. Ambika Prasad Research Foundation, India. DOI: 10.5281/zenodo.13997051.

Sharma N., Kumar N., James J., Kalia S., Joshi S. 2023. Strategies for successful acclimatization and hardening of *in vitro* regenerated plants: challenges and innovations in micropropagation techniques. Plant Science Today 10(sp2): 90–97. DOI: 10.14719/pst.2376.

Sharma U., Kataria V., Shekhawat N.S. 2017. In vitro propagation, ex vitro rooting and leaf micromorphology of *Bauhinia racemosa* Lam.: a leguminous tree with medicinal values. Physiology and Molecular Biology of Plants 23(4): 969–977. DOI: 10.1007/s12298-017-0459-2.

Shekhawat J.K., Rai M.K., Shekhawat N.S., Kataria V. 2021. Synergism of *m*-topolin with auxin and cytokinin enhanced micropropagation of *Maytenus emarginata*. In Vitro Cellular and Developmental Biology – Plant 57(3): 418–426. DOI: 10.1007/s11627-020-10132-6.

Shekhawat M.S., Manokari M. 2017. Comparative foliar micromorphological studies of in vitro and field transferred plants of *Morinda citrifolia*. Acta Botanica Hungarica 59(3–4): 427–438. DOI: 10.1556/034.59.2017.3-4.8.

Singh A., Yadav S., Rai M.K. 2026. Somatic embryogenesis, secondary somatic embryogenesis and plant regeneration in *Dillenia indica* L., an underutilized fruit tree. In Vitro Cellular and Developmental Biology – Plant. DOI: 10.1007/s11627-025-10620-7.

Taha L.S., Ibrahim E.A., Youssef N.M., El-Sayed I.M. 2018. *In vitro* culture development and secondary metabolites of *Dillenia indica* tree. American Journal of Plant Physiology 13(1): 44–52. DOI: 10.3923/ajpp.2018.44.52.

Vibha J.B., Shekhawat N.S., Mehandru P., Dinesh R. 2014. Rapid multiplication of *Dalbergia sissoo* Roxb.: a timber yielding tree legume through axillary shoot proliferation and ex vitro rooting. Physiology and Molecular Biology of Plants 20(1): 81–87. DOI: 10.1007/s12298-013-0213-3.

Vujović T., Ružić D., Cerović R. 2012. *In vitro* shoot multiplication as influenced by repeated subculturing of shoots of contemporary fruit rootstocks. Horticultural Science (Prague) 39(3): 101–107. DOI: 10.17221/208/2011-hortsci.

Yadav S., Singh A., Mohan M., Das S.N., Rai M.K. 2025. In vitro propagation, phytochemical analysis and assessment of antioxidative potential of micropropagated plants of *Tecomaria capensis* (Thunb.) Spach. South African Journal of Botany 185: 16–23. DOI: 10.1016/j.sajb.2025.07.045.

Yazan L.S., Armania N. 2014. *Dillenia* species: a review of the traditional uses, active constituent and pharmacological properties from pre-clinical studies. Pharmaceutical Biology 52(7): 890–897. DOI: 10.3109/13880209.2013.872672.

Zalt N., Yasmin S., Hamakhan A.M., Polat Ş., Yu H., Ge C. et al. 2024. Effect of auxins and activated carbon on *in vitro* rooting of two blueberry cultivars (*Vaccinium corymbosum*). Journal of Horticultural Research 32(2): 75–80. DOI: 10.2478/johr-2024-0016.