PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.8352

eISSN 2348-1900

RESEARCH ARTICLE

Cytokinin improves *in vitro* organogenesis, biological activities and blooming in *Vitex negundo* L. - An endangered medicinal plant

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Received: 18 March 2025; Accepted: 18 July 2025; Available online: Version 1.0: 10 October 2025

Cite this article: Shweta C, Alok B, Gunjan G. Cytokinin improves *in vitro* organogenesis, biological activities and blooming in *Vitex negundo* L. - An endangered medicinal plant. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.8352

Abstract

Vitex negundo L., an aromatic species, is used worldwide to cure several illnesses due to the presence of pharmacologically important metabolites in it. Cytokinin's show plant growth promoting activity, so can be exploited in micropropagation of medicinal plants. The present study was aimed to assess the potential of cytokinin in improving *in vitro* organogenesis, biological activities and blooming in *V. negundo*. *V. negundo* offers various medicinal benefits due to its rich composition of active compounds. However, its conservation is threatened due to over-exploitation, necessitating optimized micropropagation protocols for sustainable use. Standardized and reproducible micropropagation systems are crucial for conservation and medical applications. To induce the axillary bud formation, explants were placed on Murashige and Skoog (MS) basal medium enriched with different plant growth regulators (PGRs) as 6-benzylaminopurine (BAP), diphenyl urea (DPU), thidizuron (TDZ) and meta-topolin (mT), individually or in combinations. The newly grown shoots were planted in half-strength MS medium supplemented naphthalene acetic acid. After sufficient root development, the rooted shoots were planted in small poly-stacks comprising of garden soil, sand and compost (1:1:1). Plantlets from *in vitro* cultures were successfully transplanted into the soil. Direct shoot regeneration and blooming of *V. negundo* were primarily induced by cytokinin. The optimal concentration of cytokinins for shoot regeneration was found to be 2.0 mg L^{-1} mT and 1.5 mg L^{-1} TDZ in MS medium. After 37 days of cultivation, the nodal explants showed a 100 % shoot regeneration rate at this concentration. The study revealed the potential of cytokinin in improving the biological activity of *V. negundo*.

Keywords: acclimatization; cytokinin; micropropagation; meta-topolin; naphthalene acetic acid; thidizuron

Introduction

Plants are an excellent source of biologically active compounds of pharmaceutical significance. There is renewed interest in natural medicines obtained from various plant parts (1). However, the increasing use of herbs for healthcare has imposed great threat to the endangered plant species and natural resources (2). Many pharmaceutical companies largely depend on wild medicinal plants as raw materials for the extraction of medically important compounds (3). The genetic diversity of medicinal plants is depleting at an alarming rate due to their over -exploitation and ruinous harvesting race for medicines. Hence, there is urgent need to conserve, cultivate and sustain the use of medically important plants (4). Several biotechnological tools and techniques are used to conserve the rare and threatened plants (5) to enhance their multiplication through *in vitro* regeneration (6), genetic transformation and production of secondary metabolites using plants as bioreactors (7).

Cytokinin's (CKs) are phytohormones that show wide-range actions on plant growth and development, so are used for improving the plant micropropagation (8). Their coordinated regulatory effects and cross-talk interactions with other phytohormones and signalling networks are highly sophisticated, eliciting and controlling the varied biological processes at cellular to organismal levels. CKs are frequently used for biotechnological manipulation in plants to enhance their regeneration. The hormone-induced changes in phytochemical profile increase the therapeutic metabolite production. Many efforts have been made to develop suitable culture protocols for many rare and endangered plant species. Many potential commercial species lack effective *in vitro* strategy for promoting biomass propagation. Hence, cytokinin-induced organogenesis has become a handy tool to increase bud regeneration rate (9). CK signalling is a potential target for enhancing future shoot regeneration efficiency, as it activates the shoot progenitor at later stages and allows chromatin to maintain shoot identity genes at priming stage. CKs also have important regulatory role in plant growth. For instance, the vegetative phase changes in *Arabidopsis thaliana* through miR172/TOE1-TOE2 module, development and environmental responses of plants through CKRs (cytokinins response) and shoot branching regulation in *Pisum sativum* through SMXL/D53 strigolactone signalling repressors and up-regulation of PsSMXL7/D53 transcripts (10).

V. negundo L. (Verbenaceae), commonly known as 'Nirgundi', is an aromatic deciduous shrub (4-5 m) found mostly in warmer zones at an altitude of 1500 m in outer region of Himalayas and some districts of Himachal Pradesh (11) and is used to cure

several illnesses worldwide. The plant is a panacea of herbal formulations with several of its metabolites exhibiting significant pharmacological properties. The plants are reported to possess several biological activities, including the improvement in receptive and retentive power of mind. Flavonoids, iridoids, terpenes and steroids are the major class of compounds isolated from *V. negundo* (12). Essential oils extracted from its leaves are used to improve skin complexion (2). Vitexin compound 1 (VB-1) has shown hair growth-promoting effects, indicating its potential as a new therapy for alopecia treatment (13). Another metabolite methyl 3-(2-5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl) ethyl) benzoate, found in the plant, is used to treat rheumatism (12) by the mechanism of radical quenching and another novel compound tris (2,4-di-tert-butylphenyl) phosphate helps in relieving the pain, chronic bronchitis and cold. Two bioactive compounds 22,23-dihydro-α-spinasterol-β-D-glucoside and salicylic acid are used as astringent, febrifuge, sedative, tonic and vermifuge. Chloroform extract from the seeds yielded triterpenoids (14). It shows anti-inflammatory activity and plant possesses potent mosquito repelling activity (15) against *Aedes aegypti*, *Culex quiquefasciatus* and *C. tritaenirrhynchus* (16). The methanolic extract of *V. negundo* has anti-bacterial activity against *E. coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Pseudomonas aeruginosa,* antifeedant activity against *Spodoptera litura* and *Achoeajanata* (17). A few previous attempts at *V. negundo* direct *in vitro* regeneration have been made (18), but none attempted the optimization of various plant growth regulators and repeated the generation of plantlets from regenerated plants (19). Therefore, the present study was aimed to standardize the impact of cytokinin on *in vitro* organogenesis, its biological activity and blooming in *V. negundo*.

V. negundo shows a wide range of medicinal benefits, such as reducing inflammation, fighting oxidation, acting against microbes, protecting the liver and combating cancer. These effects come from its rich supply of active compounds like flavonoids, alkaloids, glycosides, phenolics and essential oils. Recent research has also pointed out its antibacterial and antibiofilm abilities. Additionally, it is gaining attention for its potential in treating breast cancer, due to presence of cytotoxic flavonoids like casticin. Despite its therapeutic significance, large-scale exploitation of wild populations has raised concerns over its conservation, making *in vitro* propagation an essential alternative. Past studies have reported success in micropropagation using nodal and leaf explants with different combinations of plant growth regulators. Yet, issues like low shoot multiplication rates, poor rooting efficiency and inconsistent acclimatization remain. Moreover, there is a lack of standard protocols and limited molecular analysis of regenerated plants to ensure genetic correctness. Techniques like using bioreactors, enhancing metabolites with elicitors and integrating nanobiotechnology are still not widely explored. These gaps highlight the need for an optimized and reproducible micropropagation system that can aid both conservation efforts and sustainable use of *V. negundo* for medical purposes.

Materials and Methods

Collection and disinfection of explant material

Healthy disease-free explants of *V. negundo* were procured from the herbal garden of the University of Gautam Buddha (GBU) campus. Mature 18th month old mother plants, maintained in the herbal garden at GBU, were used to collect explant material. Apparently healthy branches were carefully selected to collect 4-5 cm long twigs. The leaves were removed and twigs washed with liquid hand soap in a 500 mL plastic container, followed by tap water washing for 30 min and then thoroughly rinsed in detergent (Tween-20) solution for 5-10 min. The plant material was surface disinfected with 0.1 % $HgCl_2$ for 3-5 min and thoroughly washed with sterile distilled water.

Preparation of culture media for shoot induction

The explants were inoculated on Murashige and Skoog (MS) basal medium, which consists of macronutrients, micronutrients, vitamins and other essential components. The MS basal medium was supplemented with different plant growth regulators (PGRs) to induce shoot bud formation and proliferation. The cytokinin treatments used were 6-benzyl amino purine (BAP), diphenyl urea (DPU), thidizuron (TDZ) and meta-topolin (mT), which were applied separately at three concentrations (1.0, 1.5 and 2.0 mg L^{-1}). Additionally, naphthalene acetic acid NAA was used as an auxin treatment at a concentration of (1.0 mg L^{-1}).

The experiment was designed as a completely randomized design (CRD) with three replications, where each treatment had 10 explants per replication, making a total 30 explants per treatment. The explants were inoculated on the MS basal medium supplemented with the different PGRs and combinations and incubated at 25 ± 2 °C with a 16-hr photoperiod. The cultures were sub-cultured every 4 weeks to maintain the shoot induction and proliferation. For shoot development, the induced shoots were transferred to the MS basal medium supplemented with combination treatments, including mT (2.0 mg L^{-1}) + NAA (1.0 mg L^{-1}) and TDZ (1.5 mg L^{-1} each) + NAA (1.0 mg L^{-1}). These cultures were also incubated at 25 ± 2 °C with a 16-hr photoperiod and sub-cultured every 4 weeks to promote shoot elongation and rooting.

Culture media for root induction

To induce roots in micro-cuttings of *in vitro* regenerated *V. negundo* shoots, a half-strength MS medium was used with two different concentrations of NAA (1.0 and 2.0 mg L^{-1}). The medium was solidified with 0.8 % agar (w/v) and the pH was adjusted to 5.7 using 0.1N NaOH and/or 0.1N HCl before autoclaving at 121 °C for 15 min. The cultures were incubated at a temperature of 27 °C and 60-70 % relative humidity under a 16-hr photoperiod.

Acclimatization and hardening

After developing a considerable root system, the regenerated explants were transferred to soil. The plantlets were carefully removed from the culture phyta-jars, which were 250 mL and/or 500 mL in size. The roots were gently rinsed under running tap water to remove any agar residue. The plantlets were then placed in small plastic pots (approximately 5-7 cm in diameter) filled with mixture of sand, compost and soil in a 1:1:1 ratio (by volume or weight). To maintain high humidity and prevent sudden dehydration, the pots were covered with polythene bags. The interior of the bags was sprayed with water every 24 hr to keep the environment humid. After 7 days, the plantlets were removed from the polythene bags and relocated to a test field. This process was repeated three times in a completely randomised design to ensure reliable results. The successful acclimatization plantlets were then transferred to larger pots or direct to the field, where they continued to grow and thrive. The plant survival in the field was 92 %.

Statistical analysis

All the experiments were conducted with three replicates and each treatment had 10 explants per replication, making a total 30 explants per treatment. To ensure the reliability of the results, the data were statistically analysed using the NTSYS-pc version 2.1 (Numerical Taxonomy and Multivariant Analysis System) (20). The data were subjected to analysis of variance (ANOVA) to determine significant differences between treatments. The means and standard errors were calculated for each treatment and the result were presented as mean ± standard error. This statistical approach allowed us to identify significant differences between treatments and to draw conclusions about the effects of different variables on the experimental outcomes.

Results and Discussion

In this study, we investigated the effects of different growth regulators at various concentrations or in combinations on the regeneration of shoots from nodal explants of *V. negundo in vitro*. We used MS medium supplements with four different growth regulators: BAP, TDZ, DPU and mT, each at three concentrations (1.0, 1.5 and 2.0 mg L^{-1}). The results of the experiment are presented in Table 1 (Fig. 1-4). After 37 days of cultivation, the highest average number of shoots was recorded in MS medium supplemented with 2.0 mg L^{-1} mT and 1.5 mg L^{-1} TDZ, with 100 % shoot regeneration. The highest response for shoot expansion was observed in MS medium with 2.0 mg L^{-1} mT, with an average number of shoots per explant and the highest number of shoot length per explant. In contrast, the medium with 1.0 mg L^{-1} BAP showed the lowest percentage of shoot regeneration (40 %). The medium with 1.0 mg L^{-1} DPU showed an average fewer number of shoots and shoot length per explant, with 80 % of cultures responding (Fig. 5).

For root initiation, half-strength MS medium was used. The result showed that root induction was relatively low in MS_0 medium (MS media without PGRs). Regenerated shoots (2.5-3.5 cm) were transferred to half strength MS medium supplemented with NAA at two different concentrations (1.0 mg L^{-1} and 2.0 mg L^{-1}) for root induction. The result showed that 98 % root induction was achieved when shoots were transferred to half strength MS medium supplemented with 1.0 mg/L NAA (Fig. 6). In contrast, the percentage of root induction was lower (less than 98 %) when 2.0 mg L^{-1} NAA was used (Table 2) (Fig. 7).

To enhance shoot development, we investigated the effects of different mixtures of cytokinins and auxins. Specially, we used two combinations: 2.0 mg L^{-1} mT + 1.0 mg L^{-1} NAA and 1.5 mg

Table 1. Effect of three different concentrations (1.0, 1.5 and 2.0 mg L^{-1}) of BAP, DPU, TDZ and mT on shoot regeneration of *V. negundo* nodal explant after 37 days and mT showed the best response amongst all PGRs

PGRS (mg L^{-1})	Concentration of PGRs (mg L^{-1})	No. of explant inoculated/ phyta jars	Explants survival (no.) out of 5 explants inoculated in a phyta jar	Shoot regeneration (%)	No. of shoots (mean ± SE)	Mean shoot length (cm)
Control	0	5	0	0	0	0
BAP	1.0	5	3	60	3 ± 0.57	1.88 ± 0.33
		5	4	80	3.5 ± 0.5	3.3 ± 0
		5	2	40	2.6 ± 0.66	2.96 ± 0.66
	1.5	5	4	80	3 ± 0.40	3.05 ± 0.47
		5	2	40	3.33 ± 0.33	2.93 ± 0.33
		5	3	60	4 ± 0	4.85 ± 0.5
	2.0	5	5	100	3.5 ± 0.5	4.7 ± 0.5
		5	3	60	3.66 ± 0.33	3.43 ± 0.33
		5	3	60	3 ± 0.40	3.8 ± 0.25
DPU	1.0	5	4	80	3.5 ± 0.5	3.7 ± 0.5
		5	4	80	2.33 ± 0.88	3.23 ± 0.33
		5	5	100	2.5 ± 0.5	4.05 ± 0
	1.5	5	3	60	3.33 ± 0.33	3.7 ± 0.33
		5	3	60	3 ± 0.57	3.56 ± 0.57
		5	2	40	3 ± 0.57	4.06 ± 0
	2.0	5	4	80	3.33 ± 0.33	3.86 ± 0.33
		5	5	100	4 ± 0	3.35 ± 0
		5	3	60	4 ± 0	4.3 ± 0
TDZ	1.0	5	4	80	4 ± 0.40	4.26 ± 0
		5	5	100	4.4 ± 0.24	4.56 ± 0.20
		5	4	80	4.16 ± 0.30	4.15 ± 0.30
	1.5	5	5	100	4.6 ± 0.24	4.38 ± 0.20
		5	5	100	5.4 ± 0.24	4.44 ± 0.37
		5	5	100	5.16 ± 0.30	5.06 ± 0.16
	2.0	5	5	100	4.83 ± 0.54	4.83 ± 0.80
		5	5	60	4.85 ± 0.40	4.57 ± 0.28
		5	3	80	3.5 ± 0.42	4.1 ± 0.36
MT	1.0	5	4	80	4.8 ± 0.37	5.4 ± 0.77
		5	4	80	5 ± 0.44	5.58 ± 0.49
		5	5	100	5.16 ± 0.30	5.66 ± 0.22
	1.5	5	4	80	5 ± 0.44	5.81 ± 0.22
		5	5	100	4.8 ± 0.30	5.88 ± 0.21
		5	5	100	5.6 ± 0.24	5.78 ± 0.24
	2.0	5	5	100	5.16 ± 0.30	5.95 ± 0.16
		5	5	100	5.57 ± 0.20	6.17 ± 0
		5	5	100	5.85 ± 0.14	6.05 ± 0.14

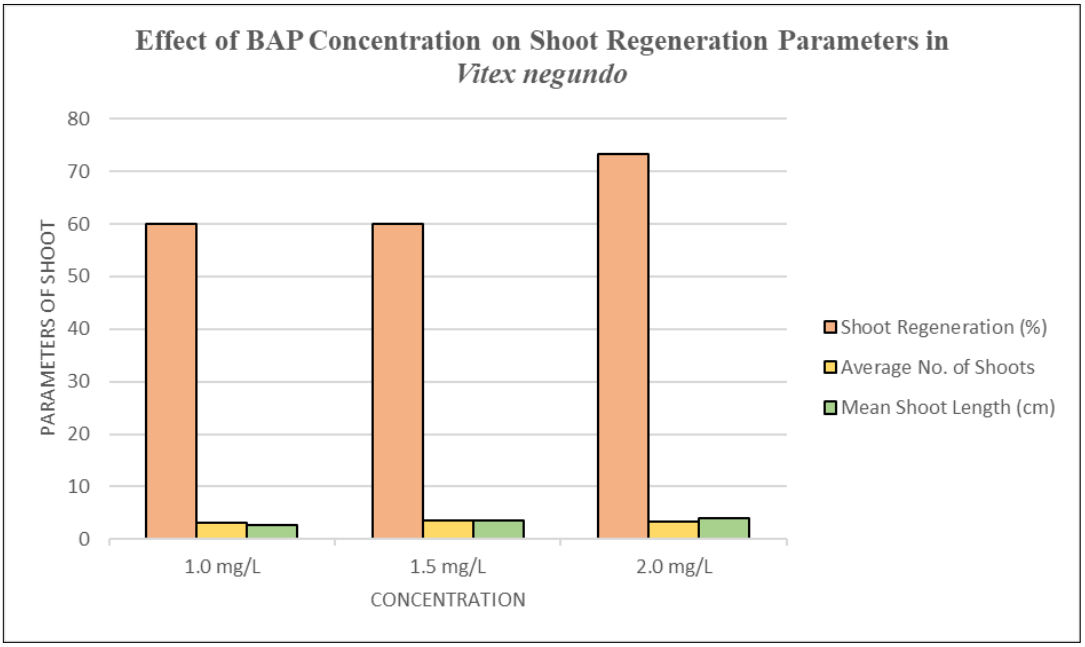

Fig. 1. Effect of BAP concentration on shoot regeneration parameters in *Vitex negundo*.

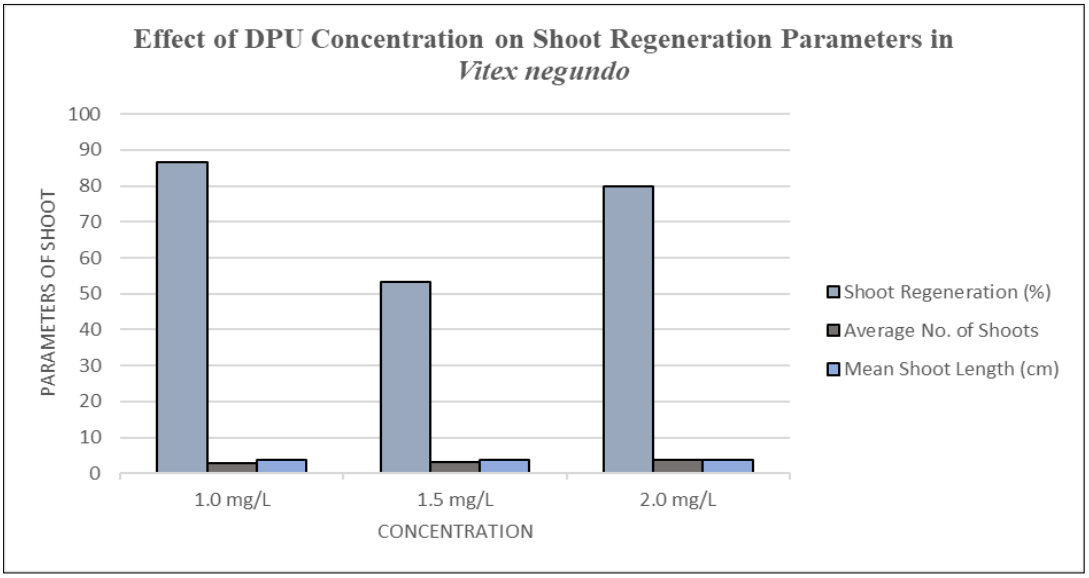

Fig. 2. Effect of DPU concentration on shoot regeneration parameters in *Vitex negundo*.

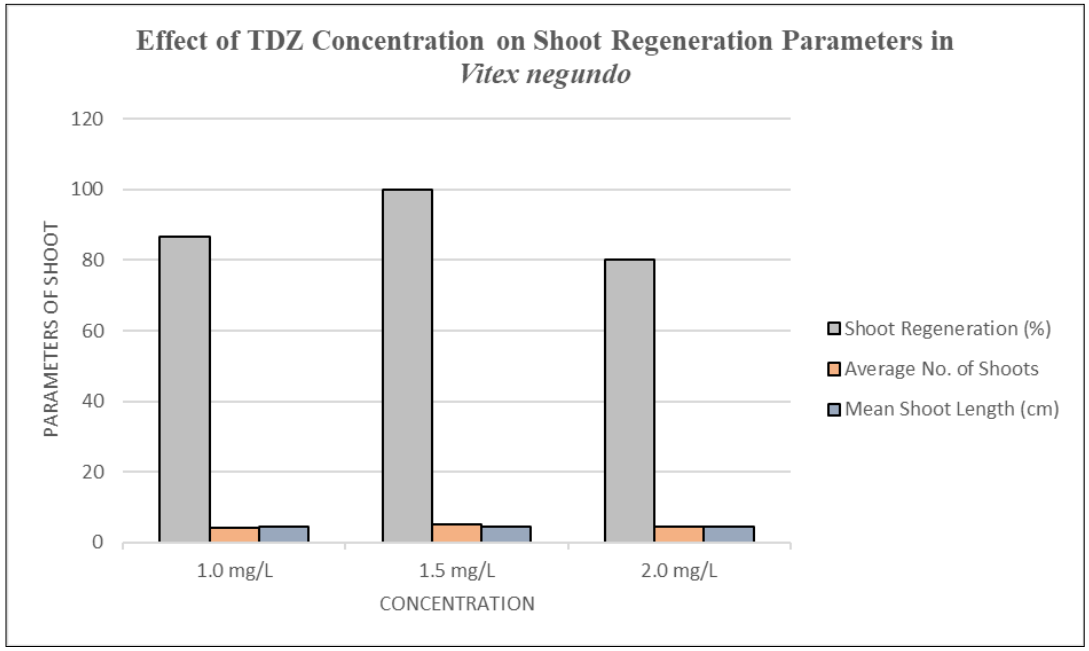

Fig. 3. Effect of TDZ concentration on shoot regeneration parameters in *Vitex negundo*.

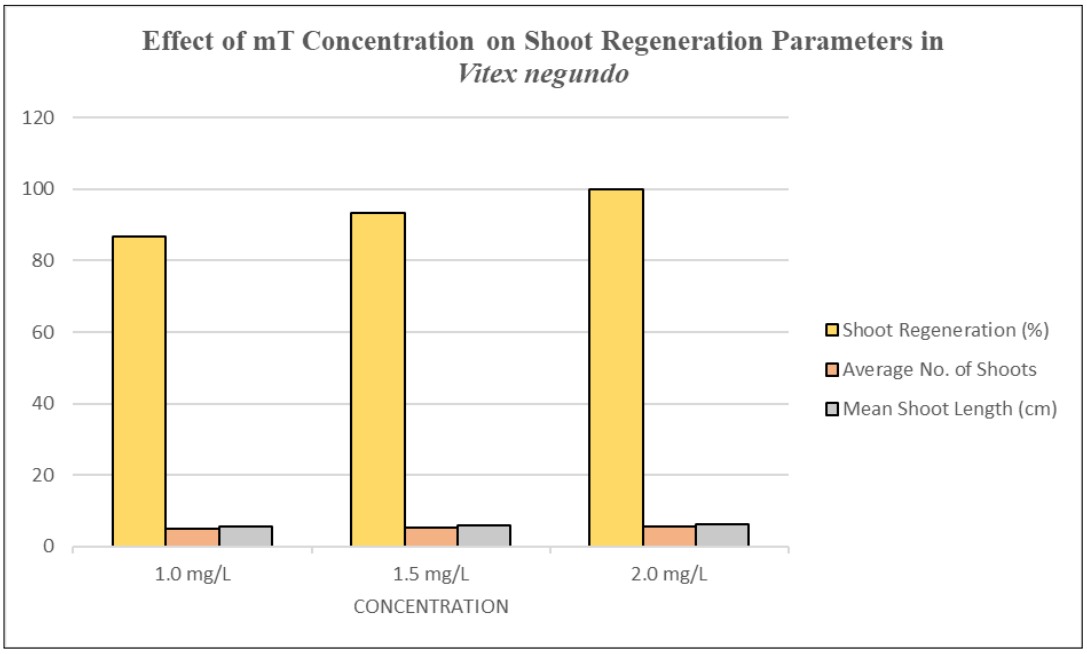

Fig. 4. Effect of mT concentration on shoot regeneration parameters in *Vitex negundo*.

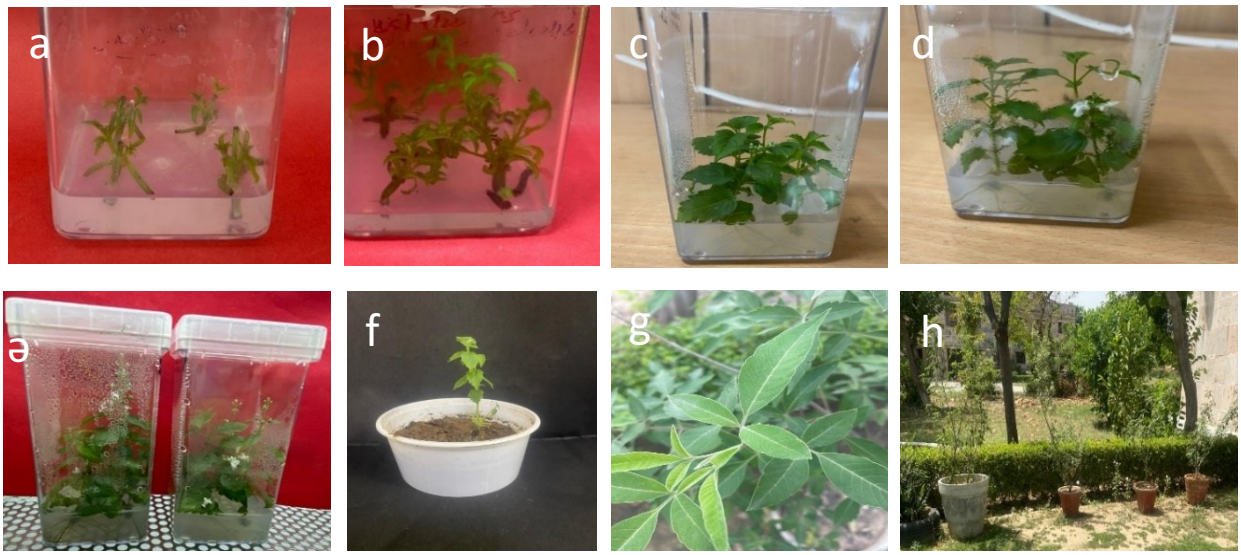

Fig. 5. Growth and *in vitro* blooming of *V. negundo*; (a) Shoot initiation from nodal explants of *V. negundo* on MS medium; (b) Shoot proliferation on MS medium; (c-d) Shoot elongation on MS medium; (e) *In vitro* blooming of *V. negundo*; (f) Hardening of micro-propagated plants; (g) Close-up of micro-propagated plants; (h) Field grown micro-propagated plants.

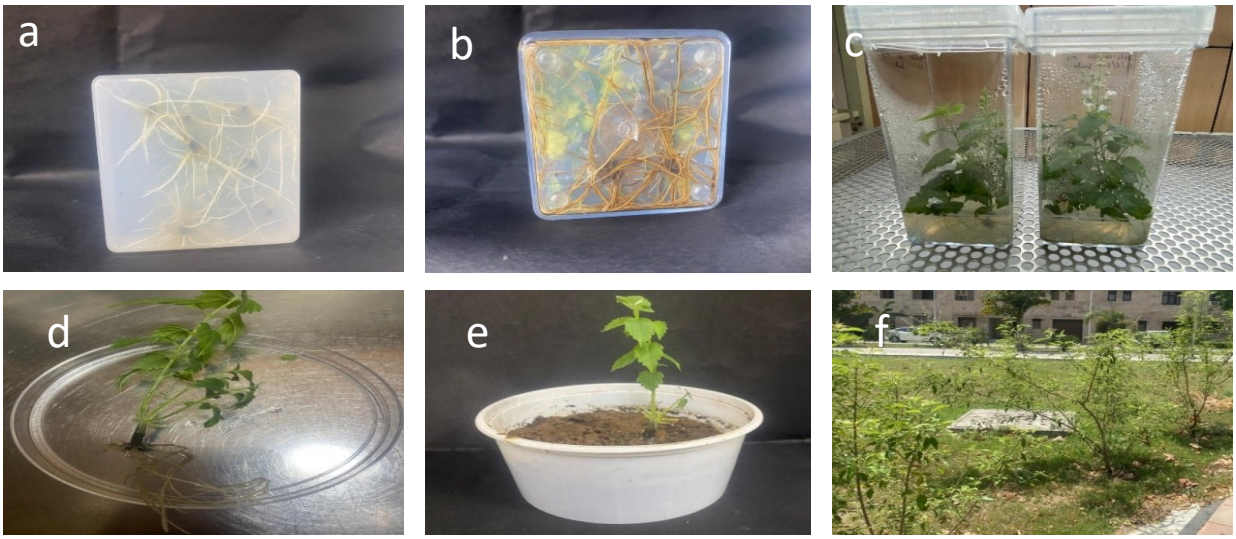

Fig. 6. Growth of *V. negundo* and *in vitro* rooting of *V. negundo*; (a) Initiation of *in vitro* rooting from shoots of nodal explants; (b) *In vitro* rooting from shoots; (c) *In vitro* rooting and shooting of *V. negundo*; (d) Close-up of *in vitro* rooting and shooting of explant; (e) Hardening of micro-propagated plant; (f) Field grown of micro-propagated plants.

Table 2. Effect of auxin NAA on *in vitro* root induction in tissue culture raised from shootlets of *Vitex negundo* after 21 days of culture

PGR NAA (mg L^{-1})	Concentration of NAA (mg L^{-1})	No. of root regenerated (%)	Average no. of roots (mean± SE)	Average length of root (cm)
1.0	1.0	60 %	5.66 ± 0.33	7 ± 0.11
	1.0	70 %	6 ± 0	7.03 ± 0.08
	1.0	80 %	7 ± 0.57	6.5 ± 0.25
2.0	2.0	30 %	4 ± 0.57	6.13 ± 0.52
	2.0	50 %	3.33 ± 0.33	6.03 ± 0.08
	2.0	40 %	2.66 ± 0.66	5.93 ± 0.37

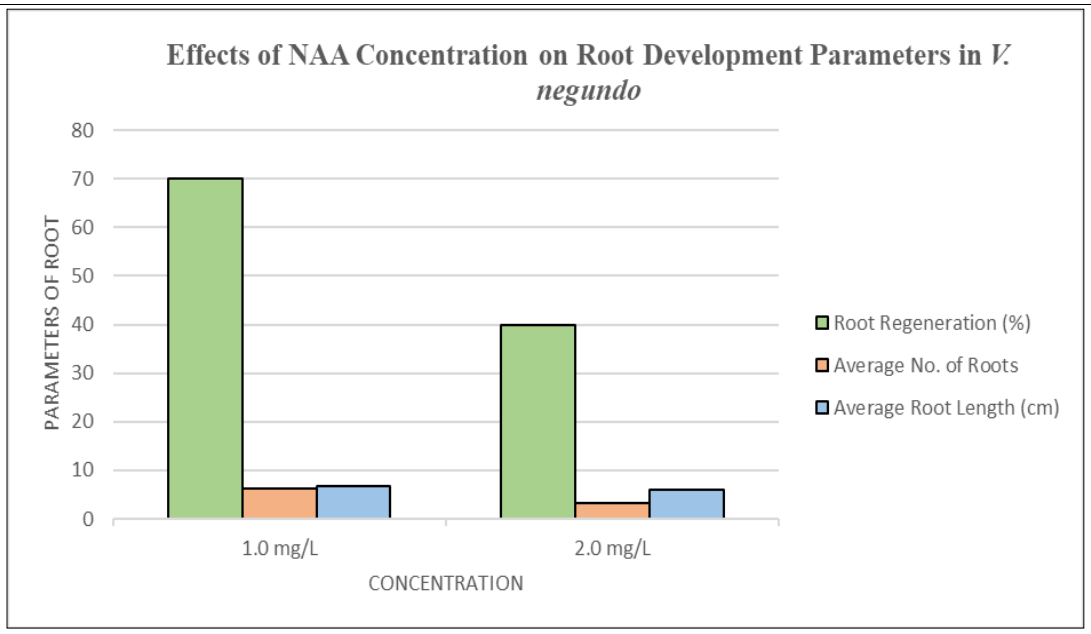

Fig. 7. Effects of NAA concentration on root development parameters in *V. negundo*.

L^{-1} + 1.0 mg L^{-1} NAA. The result presented in Table 3 (Fig. 8) shows that the combination of 2.0 mg L^{-1} mT + 1.0 mg L^{-1} NAA was the most effective for enhancing multiple shoots. This combination resulted in 98.7 % shoot formation, with an average shoot length. Furthermore, 100 % of the shoots responded to this treatment, with an average shoot length (Fig. 5).

In this study, we used regenerated shoots were as explants to induce blooming. We found that blooming occurred when medium was supplemented with cytokinins (BAP, DPU, TDZ and mT) or in combination with NAA. However, the blooming was not uniform in each trial. We observed that blooming failed to occur when the medium was fortified with any one of the cytokinins (BAP, DPU, TDZ and mT) alone or with auxin alone. However, when the MS medium was supplemented with different concentrations of cytokinins (BAP, DPU, TDZ and mT) in combination with auxin (NAA), the shoots started developing

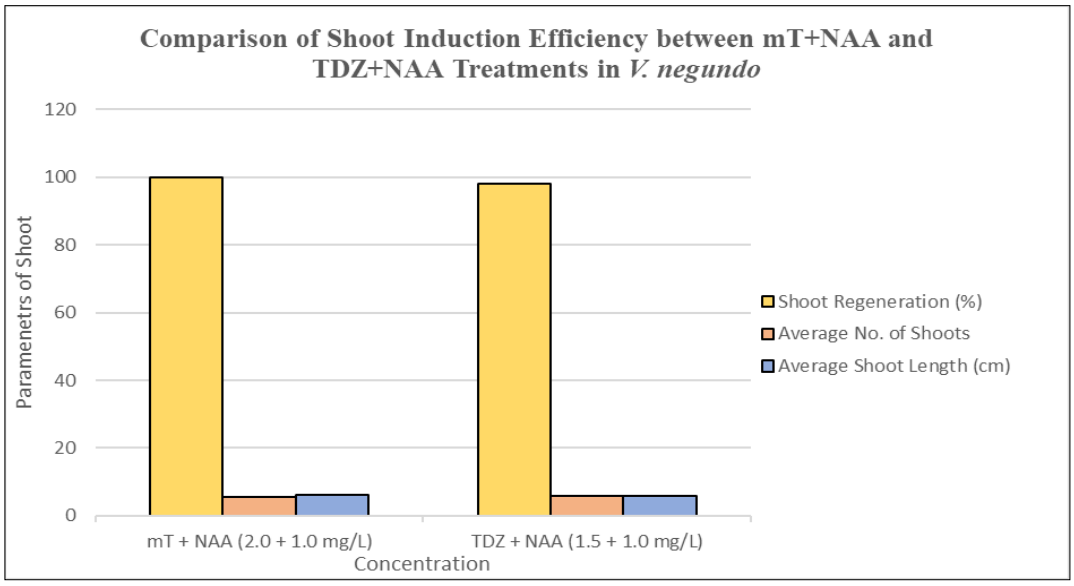

Fig. 8. Comparison of shoot induction efficiency between mT + NAA and TDZ + NAA treatments in *V. negundo*.

Table 3. Effect of mixtures of cytokinin and auxin on *in vitro* shoot induction in tissue culture raised plantlets of Vitex negundo after 15 days of culture

PGR	Concentration (mg L^{-1})	No. of shoot regenerated %	Average no. of shoots (mean ± SE)	Average length of shoots (cm)
mT + NAA	2.0 + 1.0	100 %	5.57 ± 0.20	6.05 ± 0
TDZ + NAA	1.5 + 1.0	98 %	5.85 ± 0.14	5.97 ± 0.16

inflorescence at the shoot apex. In our study, we found that the combination of mT and NAA was the most effective for promoting flower bud formation and blooming. Blooming occurred after 10 days in the presence of this combination (Fig. 5). This suggests that the interaction between mT and NAA plays a crucial role in inducing blooming in regenerated shoots of *V. negundo*.

This study developed an *in vitro* protocol for the proliferation of shoots, root induction and effective adaptation of the medicinal herb *V. negundo* L. The protocol involved surface sterilization of explants with 0.1 % $HgCl_2$ for 5 minutes, resulting in over 94 % explants being free of surface contaminants with minimal tissue injury. The explants were then cultivated to investigate clonal propagative effectiveness, explants were cultivated on MS medium with various concentrations and mixtures of cytokines and auxin to investigate clonal propagative effectiveness, with the greatest response observed at 2.0 mg L^{-1}mT. Regenerated shoot tips were induced to root in half-strength MS media supplemented with two different doses NAA and the optimal rooting was achieved with half-strength MS medium containing 1.0 mg L^{-1} NAA, yielding a high number of healthy roots, thus establishing an efficient *in vitro* protocol for large-scale propagation of *V. negundo* (Fig. 6).

The present study found that a combination of 2.0 mg L^{-1} mT and 1.0 mg L^{-1} NAA was the most effective for nodal segment and shoot tip culture of *V. negundo*, with the highest concentration of 2.0 mg L^{-1} mT yielding the largest number of shoots per explant on average. This result is consistent with previous studies, such as those on Chrysanthemum (21) and shoot multiplication in other plants, where mT at 2.0 mg L^{-1} was found to have a positive impact (22). Additionally, BAP at 2.0 mg L^{-1} was found to be the most effective concentration for shoot tip culture in pear plants (23), while the combination of mT at 2.0 mg L^{-1} and NAA at 1.0 mg L^{-1} was found to be the most effective for regeneration via nodal induction.

The results of this study are also in line with other research on plant tissue culture, where the use of auxins and cytokinins has been found to be effective for shoot proliferation. For example, the combination of 9.0 M BAP and 0.5 M indole-3-acetic acid was found to be the most effective for shoot-tip cultures of *Pyrus elaeagrifolia Pallas* (24), while NAA was found to be more effective than IAA or IBA for producing multiple shoots in rose genotypes (25). Similarly, the use of 2, 4-D and BAP was found to be effective for shoot proliferation in *Woodfordia fruticose* (26) and the combination of BAP and IAA was found to be the most effective for shoot proliferation from shoot tips and nodal segment explants. In the present study, nodal segments were found to have a higher shooting frequency multiplication than shoot tips in *V. negundo*, highlighting the importance of explant selection for successful plant tissue culture.

Upon culturing nodal segments and shoot tips on MS media that containing BAP, DPU, TDZ and mT in different compositions, it was shown that mT outperformed other cytokinins relation to shoot multiplication. Because of differences in genotypes and explant, various authors may have come up with different conclusions. Nodal segments and shoot tips were rooted and grown into complete plantlets. Root and shoot development derived from shoots are particularly critical for tissue culture-derived shoot establishment (27). To find the optimal root induction strategy, many tests had been completed using a half-strength MS medium added with different amounts of nutrients, such as auxin (NAA). However, the regenerated shoots were discovered to be effective when rooted on MS media added with 0.5 mg L^{-1} IBA to induce root from *in vitro* grown shoots of *V. negundo* was investigated in the previous study (28). Half-strength MS medium added with 0.5 mg L^{-1} IBA was used to grow rooting shoots. Nevertheless, in this inquiry NAA was used alone for rooting. In both half-strength, MS mediums with 1.0 mg L^{-1} NAA, the optimal concentration for root development and proliferation was obtained.

This study also reports the role of four cytokinins (BAP, DPU, TDZ and mT) for inducing shoots from explants. In the present study, it was found that the shoot regeneration was negatively impacted by high concentrations of mT and NAA (29). In this study, it is also found that auxin and cytokinin combinations (mT + NAA) and (TDZ + NAA) stimulate blooming. Meta-topolin was superior overall other cytokinin for shoot regeneration but also for rooting (30).

Conclusion

The micro-propagation method developed for *V. negundo* in this study was highly efficient, with rapid shoot multiplication and rooting. Cytokinins, a class of plant hormones, play a crucial role in plant growth and development and their importance in micropropagation has become increasingly clear. The results showed that meta-topolin (mT) outperformed other cytokinins in enhancing in shoot multiplication, shoot growth and rooting. This makes mT a promising candidate for *in vitro* propagation of other *Vitex species*. The study also found that combining auxin and cytokinin (mT + NAA) and (TDZ + NAA)) stimulated blooming. After a brief hardening period, micro propagated plantlets of *V. negundo* showed a remarkable 98 % survival rate when transferred to the soil. Overall, mT superior to other cytokinins for both shoot regeneration and rooting. This study demonstrated the effectiveness of using four cytokinins (BAP, DPU, TDZ and mT) to induce shoots from explants. The developed procedure provides a reliable strategy for the conservation and large-scale propagation of this valuable aromatic medicinal plant. Cytokinin-based micro propagation offers a promising technique for the large-scale multiplication and preservation of *V. negundo*, ensuring its availability for future generations.

Acknowledgements

The authors express their gratitude to all members and the leadership of Laboratory of Plant Tissue Culture and Gautam Buddha University of Greater Noida for the work conducted in their facilities.

Authors' contributions

SC participated in writing - review & editing, data curation, conceptualization. AB carried out data curation and GG helped in writing original draft, formal analysis, data curation. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Ribeiro GJG, Rei Yan SL, Palmisano G, Wrenger C. Plant extracts as a source of natural products with potential antimalarial effects: an update from 2018 to 2022. Pharmaceutics. 2023;15(6):1638. https://doi.org/10.3390/pharmaceutics15061638
2. Cokul Raj M, Manokari M, Arumugam N, Dey A, Faisal M, Alatar AA, et al. Silicon nanoparticles mediated *in vitro* flowering and study of pollen viability in *Vitex negundo* L. Silicon. 2023;15(11):4861-71. https://doi.org/10.1007/s12633-023-02397-4
3. Noor F, Qamar MTU, Ashfaq UA, Albutti A, Alwashmi ASS, Aljasir MA. Network pharmacology approach for medicinal plants: review and assessment. Pharmaceuticals. 2022;15(5):572. https://doi.org/10.3390/ph15050572
4. Manokari M, Priyadharshini S, Shekhawat MS. Micro-structural stability of micropropagated plants of *Vitex negundo* L. Microsc Microanal. 2021;27(3):626-34. https://doi.org/10.1017/S1431927621000283
5. Sharma P, Roy M, Roy B. A review on influence of floral biology, pollination efficiency and conservation strategies of endangered medicinal plant *Rauvolfia serpentina* (L.) Benth. ex Kurz. Ann Phytomed. 2022;11(1):86-98. https://doi.org/10.54085/ap.2022.11.1.9
6. Nazir U, Gul Z, Shah GM, Khan NI. Interaction effect of auxin and cytokinin on *in vitro* shoot regeneration and rooting of endangered medicinal plant *Valeriana jatamansi* Jones through tissue culture. Am J Plant Sci. 2022;13(2):1-14. https://doi.org/10.4236/ajps.2022.132014
7. Poeaim A, Prasertkittikul N, Wangsor N, Boonmee W, Laipasu P. A new method to increase plant quantity through tissue culture on *Vitex negundo* L. Int J Agric Tech. 2022;18(5):1901-10.
8. Liu Y, Zhang M, Meng Z, Wang B, Chen M. Research progress on the roles of cytokinin in plant response to stress. Int J Mol Sci. 2020;21(18):6574. https://doi.org/10.3390/ijms21186574
9. Grzegorczyk-Karolak I, Hnatuszko-Konka K, Krzemińska M, Olszewska MA, Owczarek A. Cytokinin-based tissue cultures for stable medicinal plant production: regeneration and phytochemical profiling of *Salvia bulleyana* shoots. Biomolecules. 2021;11(10):1513. https://doi.org/10.3390/biom11101513
10. Fathy M, Saad Eldin SM, Naseem M, Dandekar T, Othman EM. Cytokinins: wide-spread signaling hormones from plants to humans with high medical potential. Nutrients. 2022;14(7):1495. https://doi.org/10.3390/nu14071495
11. Koirala N, Dhakal C, Munankarmi NN, Ali SW, Hameed A, Martins N, et al. *Vitex negundo* Linn.: phytochemical composition, nutritional analysis and antioxidant and antimicrobial activity. Cell Mol Biol. 2020;66(4):1-7. https://doi.org/10.14715/cmb/2020.66.4.1
12. Vigneswari T, Kanthimathi G, Muthulakshmi L. Superparamagnetic properties of iron oxide nanoparticles using *Vitex negundo* leaf extract by green synthesis method and its antimicrobial activity against wound pathogen. Mater Today Proc. 2023;83:306-11. https://doi.org/10.1016/j.matpr.2023.06.293
13. Luo J, Chen M, Liu Y, Xie H, Yuan J, Zhou Y, et al. Nature-derived lignan compound VB-1 exerts hair growth-promoting effects by augmenting Wnt/β-catenin signaling in human dermal papilla cells. PeerJ. 2018;6:e4737. https://doi.org/10.7717/peerj.4737
14. Tawfeeq TA, Tawfeeq AA, Eldalawy R, Ibraheem SK. Phytochemical analysis, GCMS identification and estimation of antioxidant activity of Iraqi *Vitex negundo* L. J Med Chem Sci. 2023;6(4):876-83. https://doi.org/10.26655/JMCHEMSCI.2023.4.19
15. Aswar PB, Khadabadi SS, Kuchekar BS, Rajurkar RM, Saboo SS, Javarkar RD. *In vitro* evaluation of antibacterial and antifungal activity of *Vitex nigundo* (Verbenaceae). Ethnobot Leaflets. 2009;2009(7):13.
16. Goswami S, Roy B. *Vitex negundo* L., an indigenous plant: a systematic review on traditional use, bioactives and pharmacological activities. In: Bioactives and Pharmacology of Lamiaceae. Apple Academic Press; 2023.
17. Chandramu C, Rao DM, Reddy VD. High frequency induction of multiple shoots from nodal explants of *Vitex negundo* L. using sodium sulphate. J Plant Biotech. 2003;5(2):107-13.
18. Sahoo Y, Chand PK. Micropropagation of *Vitex negundo* L., a woody aromatic medicinal shrub, through high-frequency axillary shoot proliferation. Plant Cell Rep. 1998;18(3-4):301-7. https://doi.org/10.1007/s002990050576
19. Kadhim ZK, Nayyef MN, Awadh HAA, Jaafar HM, Abdulhussein MAA. Impact of plant growth regulators and adenine sulfate on *Gardenia jasminoides* micropropagation. Plant Arch. 2020;20(2):71-5.
20. Kizilgeci F, Bayhan B, Türkoğlu A, Haliloglu K, Yildirim M. Exploring genetic diversity and population structure of five *Aegilops* species with inter-primer binding site (iPBS) markers. Mol Biol Rep. 2022;49(9):8567-74. https://doi.org/10.1007/s11033-022-07689-3
21. Firgiyanto R, Rohman HF, Azizah M, Triwidiarto C, Riskiawan HY. Effect of modified Murashige and Skoog medium on chrysanthemum tissue culture. IOP Conf Ser Earth Environ Sci. 2023;1168(1):012008. https://doi.org/10.1088/1755-1315/1168/1/012008
22. Sharma KK, Bhojwani SS, Thorpe TA. Factors affecting high frequency differentiation of shoots and roots from cotyledon explants of *Brassica juncea* (L.) Czern. Plant Sci. 1990;66(2):247-53. https://doi.org/10.1016/0168-9452(90)90210-F
23. Kotb OM, Abd El-Latif FM, Atawia AR, Saleh SS, El-Gioushy SF. *In vitro* propagation and callus induction of pear (*Pyrus communis* cv. Le-Conte). Asian J Biotechnol Genet Eng. 2020;3(2):49-58.
24. Ibrahim Ahmed Osman S, Dumanoğlu H. Using float hydroculture for acclimatization of *in vitro* plantlets in some *Pyrus* genotypes. Sci Hortic. 2023;320:112199. https://doi.org/10.1016/j.scienta.2023.112199
25. Kaur K, Kaur K, Bhandawat A, Pati PK. *In vitro* shoot multiplication using meta-topolin and leaf-based regeneration of a withaferin A rich accession of *Withania somnifera* (L.) Dunal. Ind Crops Prod. 2021;171:113872. https://doi.org/10.1016/j.indcrop.2021.113872
26. Borchetia S, Das SC, Handique PJ, Das S. High multiplication frequency and genetic stability for commercialization of the three varieties of micropropagated tea plants (*Camellia* spp.). Sci Hortic. 2009;120(4):544-50. https://doi.org/10.1016/j.scienta.2008.12.007
27. Abdulhafiz F, Mohammed A, Reduan MFH, Kari ZA, Wei LS, Goh KW. Plant cell culture technologies: a promising alternative to produce high-value secondary metabolites. Arab J Chem. 2022;15(11):104161. https://doi.org/10.1016/j.arabjc.2022.104161
28. Jawahar M, Ravipaul S, Jeyaseelan M. *In vitro* regeneration of *Vitex negundo* L. - a multipurpose woody aromatic medicinal shrub. Plant Tissue Cult Biotechnol. 2009;18(1):65-72. https://doi.org/10.3329/ptcb.v18i1.3263
29. Hussain SA, Ahmad N, Anis M. Synergetic effect of TDZ and BA on minimizing the post-exposure effects on axillary shoot proliferation and assessment of genetic fidelity in *Rauvolfia tetraphylla* (L.). Rendiconti Lincei. 2018;29(1):109-15. https://doi.org/10.1007/s12210-018-0667-x
30. Kucharska D, Orlikowska T, Maciorowski R, Kunka M, Wójcik D, Pluta S. Application of meta-topolin for improving micropropagation of gooseberry (*Ribes grossularia*). Sci Hortic. 2020;272:109529. https://doi.org/10.1016/j.scienta.2020.109529

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