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Synergistic Effect of Putrescine and TDZ on *in vitro* Regeneration of *Lagerstroemia speciosa* L.

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ABSTRACT: *Lagerstroemia speciosa*, an important medicinal plant known for its anti-diabetic properties due to the presence of Corosolic acid, presents challenges in commercial cultivation due to its long life cycle as a tree. This study aimed to overcome these challenges by developing a highly reproducible protocol for *in vitro* regeneration of *L. speciosa* using nodal segments or shoot tips. Various concentrations of TDZ were tested for *in vitro* shoot bud induction, followed by transfer to WPM media supplemented with cytokinin. The study found that the combination of putrescine (40µM) and mT (5 µM) in the WPM medium yielded the highest number of shoots (18.6) with an average shoot length of 13.8cm in 87.2% of the cultures after 8 weeks of incubation. Successful rhizogenesis was induced in shootlets using ½ WPM supplemented with indole-3-butyric acid (0.5 µM). Finally, the regenerated plantlets with well-developed roots were successfully acclimatized.

Keywords: Regeneration, TDZ, meta-Topolin, Putrescine.

INTRODUCTION

Lagerstroemia speciosa L., commonly known as 'Banaba' or 'queen crape-myrtle,' is a small to medium-sized deciduous tree belonging to the family Lythraceae. It holds significant importance as one of the important medicinal plants in South Asian countries (Yamada *et al.*, 2008). The plant has gained commercial interest due to the use of its crude extract in traditional systems of medicine for controlling diabetes. Corosolic acid, a compound found in Banaba leaves, has attracted wide attention due to its anti-diabetic activities as well as its anti-obesity, antioxidant, and hypoglycemic properties (Klein *et al.*, 2007; Unno *et al*., 1997).

The cultivation of *Lagerstroemia speciosa* presents challenges due to factors such as low seed germination, poor survival rate, and season-dependent response of explants. As the demand for this plant in traditional medicine increases, it becomes important to restore its natural population while meeting the growing needs of pharmaceutical industry. While the plant can be propagated through seeds or vegetative cutting, the rate of seed germination is poor, and conventional propagation methods through cuttings have proven inadequate to meet the increasing demand (Vanisree *et al*., 2004; Vijayan *et al.*, 2015).

In recent decades, plant tissue culture techniques have emerged as powerful tools for mass multiplication and propagation of medicinally important plant species. TDZ (N-phenyl-N'-1,2,3-thidiazol-5-yl-urea), an artificially modified phenyl urea, has been widely used in plant tissue culture for the regeneration of various tree species, including *Pterocarpus marsupium*, *Tecoma stans*, and *Scutellaria bornmuelleri* (Ahmad *et al*., 2021; Hussain *et al*., 2019; Gharari *et al.*, 2019). However, the use of TDZ in *in vitro* regeneration has some drawbacks, as reported in previous studies (Dewir *et al*., 2018; Huetteman and Preece 1993).

Polyamines are low molecular weight poly-cationic molecules which play an active role in plant growth and development. The addition of polyamines, such as putrescine, to culture media has been shown to induce morphogenic responses and enhanced shoot and root multiplication in various plant species such as *Mucuna pruriens*, *Polygonum multiflorum*, *Withania somnifera*, and *Cichorium intybus* (Alam *et al*., 2023; Martin-Tanguy, 2001; Park *et al*., 2022; Sivanandhan *et al.*, 2011; Bais *et al.*, 2000).

Therefore, the present study was conducted with the objective of investigating the *in vitro* regeneration potential of TDZ at different concentrations and evaluating the effect of putrescine on TDZ-exposed cultures using nodal segments or shoot tips as explants.

MATERIAL AND METHODS

A. Establishment of Aseptic culture

Isolated nodal segments or shoot tips with axillary buds were collected from a mature tree grown in the Department of Botany, AMU, Aligarh, India. The collected explants were washed thoroughly under flowing tap water for 30 min, treated with 5 % Teepol (v/v, liquid detergent) for 10 min and then finally washed under running tap water. After this, nodal

segments or shoot tips were transferred to a laminar airflow cabinet and then surface sterilization was carried out with 0.1% $HgCl_2$ (w/v) followed by thoroughly washing in sterilized double distilled water for 5 minutes. After that, aseptically excised nodal segments or shoot tips were placed on the shoot induction medium for regeneration.

B. Culture media and culture conditions

Woody plant medium (Lloyd and McCown 1980) supplemented with nutrients, 3% (w/v) sucrose and various concentrations of TDZ was used for *in vitro* induction of shoot buds in explants. The Chemicals used in the experiments were of high quality and analytical standard. The standard pH of the medium was maintained to 5.8 using 1N HCL and 1N NaOH. The solidification of the medium was done using 0.8% (w/v) agar, and then dissolved in microwave oven, followed by dispersion of medium to culture tubes before autoclaving at 121°C for 20 minutes. After inoculation of explant, culture tubes were incubated at room temperature of 25±2 °C under 16/8 (light/dark) cycle with a light intensity of 50 $\mu mol^{-2}\ s^{-1}$ supplied by white fluorescent lamps with 60-65 % of relative humidity.

C. Shoot initiation and multiplication

The culture media used for multiple shoot regeneration consist of WPM medium incorporated with TDZ at various concentrations (0.0, 1.0, 2.5, 5.0, 7.5 or 10.0 µM). Further, the best concentration of TDZ (5µM) was transferred to media containing different levels of various cytokinin *viz*. BA, mT or Kin (1.0, 2.5, 5.0, 7.5 or 10.0 µM) for enhancement of morphogenic response. Further, the optimized media was used in combination with different strengths of Putrescine (10, 20, 40, 60, 80 or 100 µM) to evaluate their effect on shoot regeneration. For adventitious shoot regeneration, WPM medium devoid of any plant growth regulator was also used to compare the effect of various plant growth regulators at different concentrations. After every 4 weeks, sub culturing was performed by transferring cultures to the similar medium. Data were recorded for shoot number, shoot length and frequency of shoot regenerated from explant.

D. In vitro rooting of shoots

For *in vitro* root induction, healthy shoots (3-4 cm) were excised from *invitro* raised culture and then inoculated on root induction media comprising WPM medium in combination with different concentrations of various auxins viz. Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and α-Naphthalene acetic acid (NAA) at various levels (0.1,0.5 or 1.0µM) . After 4 weeks, data were recorded on the percentage of rooting, root number and root length.

E. Hardening and Acclimatization

Healthy plantlets having fully expanded leaves and well- developed roots were washed with sterile distilled water to remove traces of sticky media from the surface of roots and then transfered to a thermacol cup containing sterilised Soilrite® (Keltech Energies Ltd, Bangalore). Polythene bags were used to cover thermacol cups for maintaining the required relative humidity favourable for the growth of plants. Further, the rooted plantlets grown in thermacol cup were watered every 3^{rd}-4^{th} days for the first 20 days. After this, the polythene bags were perforated and then gradually polythene bags were opened and transferred to garden soil and maintained in greenhouse under natural environment.

F. Statistical Analysis

The experiment consists of 10 replicates per treatment and was repeated three times. The data of different treatments related to percent response, number of shoots per explant and shoot length were analysed using one-way ANOVA and means were compared using Duncan's multiple range test ($p<0.05$) represented as mean± standard error (SE). All of the statistical analyses were performed using SPSS Statistics version 20.

RESULT

A. Effect of TDZ

The nodal segments or shoot tips collected from mature plants and cultured on WPM media without plant growth regulators did not exhibit any signs of bud breaking or shoot differentiation. The deposition of various alkaloids and phenolics from the explants resulted in blackening, ultimately leading to the death of plant tissues. However, successful regeneration of the cultured explants was achieved by incorporating TDZ into the WPM media, although the response in terms of shoot number and shoot length was limited. Among the concentrations tested, TDZ at 5.0µM demonstrated the highest effectiveness for nodal segments, resulting in a 44.1% response rate, with an average of 3.6 shoots and an average shoot length of 2.0 cm after 4 weeks of incubation (Table 1, Fig. 1-A). Similarly, shoot tips used in the experiment showed their best performance with TDZ at 5.0µM, exhibiting a 31.5% response rate, producing an average of 3.2 shoots with an average shoot length of 1.7 cm after 4 weeks of incubation (Table 2, Fig. 1-B).

B. Effect of Cytokinin on TDZ- induced Culture

To promote shoot proliferation and multiplication, the TDZ-exposed cultures derived from nodal segments or shoot tips were transferred to WPM media supplemented with various concentrations of different cytokinins, which play a crucial role in plant growth and development. Among the different treatments investigated, the highest regeneration frequency was observed in shoot tip explants treated with mT at 5µM, resulting in an average of 8.6 shoots with an average shoot length of 6.0cm after 8 weeks of incubation (Table-3). Additionally, BA at 7.5µM also displayed a positive effect on shoot regeneration, yielding an average of 6.1 shoots with an average shoot length of 4.5cm after 8 weeks of incubation (Table-3). In the case of KIN at 7.5µM, it produced an average of 4.7 shoots with an average shoot length of 3.5cm after 8 weeks of incubation (Table-3).

In cultures from nodal segments, mT at 5µM demonstrated the highest regeneration frequency,

resulting in an average of 12.3 shoots with an average shoot length of 8.3 cm after 8 weeks of incubation (Table 4, Fig. 1-C). Similarly, BA at 7.5µM and KIN at 7.5µM also exhibited positive effects on shoot regeneration, with maximum responses of 8.4 shoots (average shoot length of 5.7 cm) and 5.6 shoots (average shoot length of 5.1 cm) respectively after 8 weeks of incubation (Table 4).

C. Effect of Putrescine

The addition of putrescine in the optimized WPM+ mT (5µM) media positively influenced shoot multiplication and proliferation rates. The supplementation of putrescine (put) gradually enhanced the shoot multiplication capacity of the explants. Among the tested combinations, putrescine at 40µM was found to be the most effective for nodal segment cultures, exhibiting a regeneration capacity of 87.2%. This resulted in an average of 18.6 shoots with an average shoot length of 10.7 cm after 8 weeks of incubation (Table 5, Fig-1-D). However, increasing the concentration of putrescine beyond 40µM led to a gradual decrease in shoot regeneration capacity. These higher concentrations did not exhibit any positive signs of growth and proliferation after 8 weeks of incubation (Table 5).

D. In vitro rooting and acclimatization

In order to induce *in vitro* rhizogenesis, the WPM medium, both alone and in combination with auxins, was tested. When the WPM medium lacked auxins, it failed to stimulate roots even after 4 weeks of incubation. However, when the WPM medium was supplemented with various concentrations of auxins, the induction of roots from microshoots was observed. Among the tested auxin concentrations (IAA, IBA, or NAA), IBA at 0.5µM was found to be the effective, yielding a maximum response (unpublished data). The roots induced from microshoots were found to be thick and healthy.

Well-developed microshoots with 4-5 leaves and robust roots were then transferred to Soilrite® contained in thermacol cups and maintained for 4 weeks. During this period, the plantlets successfully acclimatized, displaying a remarkable survival rate of 95%.

DISCUSSION

Plant growth regulators encompass a diverse group of small organic molecules derived from various primary metabolic pathways. These regulators play a vital role in controlling plant growth and development, particularly in the context of *in vitro* regeneration. Plant hormones can act individually or in combination with other growth regulators to elicit an optimal response. TDZ, a phenylurea-substituted cytokinin analogue, has demonstrated its efficacy in promoting the growth and multiplication of several medicinal plant species (Deepa *et al*., 2018; Ahmad and Faisal 2018). Its effects are mediated through the activation of cytokinin receptors present in the plants and their subsequent signalling pathways (Nisler *et al.,* 2018). Additionally, TDZ indirectly influences plant growth by inhibiting the activity of cytokinin-degrading enzymes, such as oxidase/dehydrogenase (Nisler *et al*., 2018).

A. In vitro regeneration using Cytokinin

Naturally occurring plant hormones are small molecules synthesized in various parts of plant tissues, and they play crucial roles as regulators of plant growth and development. These hormones can act individually or synergistically to elicit different responses during plant regeneration. Propagation of mature trees through *in vitro* techniques has proven to be challenging compared to herbs and shrubs due to the physiological and biochemical changes that occur during the transition from juvenile to mature plants (Giri *et al.,* 2003; Bonga and Aderkas 1993). When explants were cultured on WPM media lacking plant growth regulators (PGRs), blackening of the explants occurred due to excessive secretion of phenolic compounds, ultimately resulting in the death of green tissues. The addition of TDZ to the WPM media partially rescued the explants from death, but issues such as tissue blackening and hyperhydricity were frequently observed in the *in vitro* cultured plantlets. Despite being a potent cytokinin-like compound, and being used in plant tissue culture for efficient *in vitro* regeneration, TDZ has been reported to induce compact shoot formation, hyperhydricity, and other negative effects in various plant species (Dewir *et al.,* 2018). Similar results have been reported in other tree species (Javed *et al.,* 2015; Ahmad and Anis 2007). The establishment of an efficient regeneration protocol depends on the type and concentration of cytokinins, their uptake and transport, as well as the endogenous levels of cytokinins present in the plant (Ahmad and Anis 2019). The effects of endogenous cytokinins are regulated through the presence of free bases, ribosides, N-linked and O-linked glycosylation, and the formation of conjugates with various sugar derivatives (Letham and Palni 1983). Some successful reports on *in vitro* regeneration of *L. speciosa* have been documented using TDZ and BA (Ahmad *et al.,* 2022; Vijayan *et al*., 2015). However, hyperhydricity and shoot tip necrosis have limited the application of these protocols. The problem of hyperhydricity can be overcome through a two-fold culture method. In the present study, TDZ-exposed cultures were transferred to secondary media containing different cytokinins at various concentrations to achieve efficient shoot regeneration and multiplication in the woody tree *L. speciosa*. This strategy of transferring cultures to secondary media for increased shoot multiplication and regeneration has been applied in many other tree species (Ahmad *et al.,* 2018; Husain and Anis 2009; Vengadesan *et al.,* 2003). After transferring the TDZ-exposed cultures to cytokinin media, the effect of meta-Topolin (mT) was found to be more pronounced as compared to BA and KIN.This may be attributed to the presence of O-glucoside in meta-Topolin, which allows for faster metabolism compared to other cytokinin used in in vitro regeneration and multiplication (Werbrouck *et al.,*1996). The effectiveness of meta-Topolin has been reported in the *in vitro* regeneration of various woody trees, such as *Caralluma umbellate* (Jayaprakash *et al.,*

2023), *Pterocarpus marsupium* (Ahmad and Anis 2019), *Syzygium cumini* (Naaz *et al.,* 2019), and *Maytenus emarginata* Shekhawat *et al.,* 2021).

B. Effect of putrescine

Polyamines (PAs) are organic polycationic molecules that function as a novel type of plant growth bio-stimulant and play crucial roles in various developmental and physiological processes in plants. These processes include DNA and protein synthesis, gene expression, cell division, and differentiation. In the context of *in vitro* conditions, polyamines are involved in diverse developmental processes such as organogenesis, somatic embryogenesis, dormancy breaking, seed germination, and senescence (Shi and Chan 2014; Tavladoraki *et al*., 2012). Furthermore, polyamines are also associated with enhanced tolerance to environmental stresses (Minocha *et al*., 2014; Hussain *et al.,* 2011). Therefore, to comprehend the role of polyamines in *in vitro* regeneration, we assessed the effects of putrescine at various concentrations on different aspects of plant growth, metabolism, and development.

The effectiveness of putrescine has been widely demonstrated in the *in vitro* regeneration of various plant species, including *Mucuna pruriens* (Alam *et al.,* 2021), *Bacopa monnieri* (Dey *et al.,* 2019), and *Citrullus lanatus* (Vasudevan *et al.,* 2017). Scaramagli *et al.* (1995) reported that putrescine (put) positively contributes to the inductive phase of cell proliferation during plant development. The addition of polyamines to optimized media significantly altered the morphogenic response of *L. speciosa*. The efficacy of putrescine may be attributed to its positive interaction in up-regulating genes involved in cytokinin, auxin, and ABA biosynthesis, while down-regulating genes associated with ethylene and gibberellic acid biosynthesis (Anwar *et al.,* 2015).

C. Rooting and Acclimatization

To achieve a successful micropropagation protocol, it is essential to establish well-developed rooted plantlets from regenerated microshoots and acclimatize them to their natural conditions.

Our results indicate that the incorporation of various concentrations of different auxins into WPM media plays a crucial role in triggering rhizogenesis. The importance of auxin in initiating root induction has been elucidated by several researchers in various plants, such as *Vitex negundo* (Ahmad and Anis 2011), *Allamanda cathartica* (Khanam *et al.,* 2020), *Bacopa monnieri* (Pramanik *et al*., 2021), and *Vanilla planifolia* (Manokari *et al*., 2021). Among the different auxins tested, IBA was found to be superior in initiating roots from *in vitro* regenerated microshoots. IBA acts as a precursor to IAA, and interconversion between the two may occur during synthesis (Bartel *et al.,* 2001). IBA is effective because it can directly act as an auxin rather than being converted to auxin precursors (Zolman *et al.,* 2000; Rashotte *et al.,* 2003). Unlike IAA, the transport of IBA does not require the activity of transporter proteins like EIR1 or AUX1 (Rashotte *et al.,* 2003). The presence of a naphthalene ring in NAA gives it different steric properties and occupies more space in the membrane compared to the indole system of IAA (Flasińsk and Hąc-Wydro 2014). NAA has more inhibitory side effects than other auxins, resulting in fewer roots induced by NAA (De clerk *et al.,* 1997). Thus, the varying effectiveness among auxins reflects possible alterations in the mechanisms of transport, uptake, and metabolism.

Furthermore, the rooted plantlets were gradually exposed to natural environmental conditions for acclimatization. No morphological variations were observed, and the regenerated plants were exact copies of the mother plant.

Table 1: Effect of different concentrations of TDZ on bud breaking from nodal explants after 30 days of incubation.

TDZ	Percent Response	Shoot Number	Shoot length
1.0	31.7±0.48^{d}	1.4±0.19^{cd}	0.74±0.15^{c}
2.5	38.6 ±0.47^{c}	2.0±0.18^{bc}	1.2±0.12^{bc}
5.0	44.1±0.35^{a}	3.6±0.34^{a}	2.0±0.25^{a}
7.5	40.5±0.33^{b}	2.6±0.26^{b}	1.6±0.22^{ab}
10	26.5±0.16^{e}	1.1±0.14^{e}	0.61±0.11^{d}

Values represent mean ± standard error of five randomly selected readings of 10 replicates per treatment in three repeated experiments. Mean sharing the same letter within columns are not significantly different (p=0.05) using Duncan's multiple range test

Table 2: **Effect of different concentrations of TDZ on bud breaking from shoot tip explants after 30 days of incubation.**

TDZ	Percent Response	Shoot Number	Shoot Length
1.0	21.4±0.37^{d}	1.2±0.15^{c}	0.66±0.12^{d}
2.5	26.4±0.30^{b}	2.2±0.43^{b}	1.2±0.25^{b}
5.0	31.5±0.29^{a}	3.2±0.37^{a}	1.7±0.35^{a}
7.5	24.7±0.40^{c}	1.7±0.14^{bc}	1.0±0.19^{bc}
10	18.2±0.24^{e}	1.0±0.11^{cd}	0.53±0.18^{de}

Values represent mean ± standard error of five randomly selected readings of 10 replicates per treatment in three repeated experiments. Mean sharing the same letter within columns are not significantly different (p=0.05) using Duncan's multiple range test

Table 3: Effect of different concentration of various cytokinins on TDZ (5µM) exposed culture obtained from shoot tips on multiple shoot regeneration in WPM medium after 8 weeks of culture

CYTOKININ			Shoot Number	Shoot length(cm)
BA	mT	KIN		
0.0			3.0±0.34^{hi}	1.6±0.31^{ij}
2.5			3.9±0.32^{efg}	2.8±0.27^{fgh}
5.0			4.8±0.35^{de}	3.6±0.20^{de}
7.5			6.1±0.14^{bc}	4.5±0.28^{bc}
10.0			3.5±0.29^{ghi}	2.4±0.26^{ghi}
	1.0		3.9±0.08^{efg}	3.0±0.50^{efgh}
	2.5		5.3±0.29^{cd}	4.1±0.23^{cd}
	5.0		8.6±0.31^{a}	6.0±0.40^{a}
	7.5		6.3±0.28^{b}	5.0±0.23^{b}
	10.0		3.6±0.49^{ghi}	2.7±0.14^{fgh}
		1.0	2.9±0.26^{hi}	1.5±0.31^{j}
		2.5	3.8±0.15^{fgh}	2.4±0.23^{ghi}
		5.0	4.5±0.26^{def}	3.3±0.17^{efg}
		7.5	4.7±0.48^{de}	3.5±0.23^{def}
		10.0	2.8±0.17^{i}	2.2±0.20^{hij}

Values represent mean ± standard error of five randomly selected readings of 10 replicates per treatment in three repeated experiments. Mean sharing the same letter within columns are not significantly different (p=0.05) using Duncan's multiple range test

Table 4: Effect of different concentration of various cytokinins on TDZ (5µM) exposed culture obtained from nodal segments on multiple shoot regeneration in WPM medium after 8 weeks of culture.

CYTOKININ (µM)			Shoot Number	Shoot length(cm)
BA	mT	KIN		
0.0			3.6±0.28^{kl}	2.0±0.19^{i}
2.5			5.3±0.23^{fgh}	3.4±0.17^{j}
5.0			6.2±0.26^{e}	5.4±0.18^{def}
7.5			8.4±0.30^{c}	5.7±0.18^{cde}
10.0			6.1±0.20^{ef}	5.0±0.14^{fgh}
	1.0		6.2±0.26^{e}	5.1±0.27^{efg}
	2.5		7.4±0.24^{c}	5.9±0.14^{cd}
	5.0		12.3±0.27^{a}	8.3±0.17^{a}
	7.5		9.6±0.43^{b}	6.8±0.08^{b}
	10.0		8.4±0.30^{c}	6.2±0.12^{c}
		1.0	4.0±0.14^{h}	2.5±0.29^{h}
		2.5	4.3±0.18^{gh}	4.6±0.37^{ghi}
		5.0	5.0±0.08^{fg}	5.7±0.14^{cde}
		7.5	5.6±0.17^{ef}	5.1±0.27^{efg}
		10.0	4.3±0.20^{gh}	4.1±0.08^{i}

Values represent mean ± standard error of five randomly selected readings of 10 replicates per treatment in three repeated experiments. Mean sharing the same letter within columns are not significantly different (p=0.05) using Duncan's multiple range test

Table 5: Effect of putrescine on *in-vitro* regeneration and multiplication from the nodal segment of *L. speciosa* cultured on WPM+ mT (5µM) after 8 weeks of culture.

Put (µM)	Shoot Number	Shoot length
10	12.5±0.27^{d}	8.8±0.11^{d}
20	14.4±0.23^{b}	9.6±0.24^{b}
40	18.6±0.25^{a}	10.7±0.14^{a}
60	13.2±0.12^{c}	9.2±0.15^{bc}
80	10.2±0.15^{e}	6.9±0.08^{e}

Values represent mean ± standard error of five randomly selected readings of 10 replicates per treatment in three repeated experiments. Mean sharing the same letter within columns are not significantly different (p=0.05) using Duncan's multiple range test

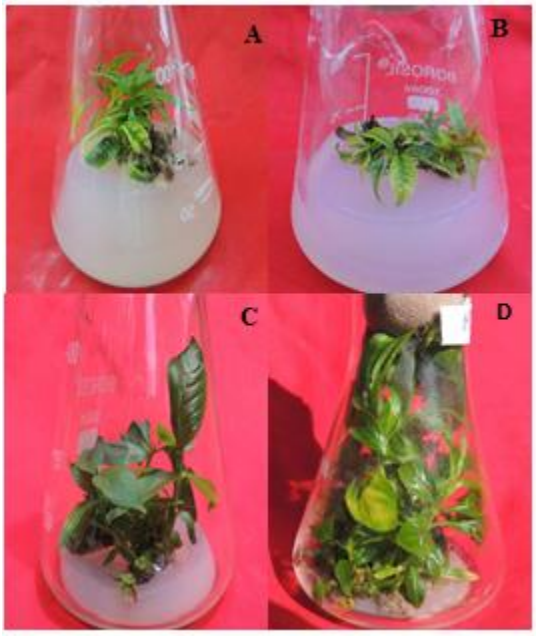

Fig. 1 (A) Shoot multiplication on TDZ (5µM) from shoot tip after 4 weeks of incubation **(B)** Shoot multiplication on TDZ (5µM) from nodal segment after 4 weeks of incubation **(C)** Shoot multiplication on mT (5µM) from nodal segment after 8 weeks of incubation **(D)** Shoot multiplication and proliferation on mT (5µM) + put (40 µM) after 8 week of incubation.

CONCLUSIONS

An efficient protocol for in vitro regeneration has been developed to enable mass propagation of Lagerstroemia speciosa L. using shoot tips or nodal segments as explants. The study also highlights the stimulating effect of putrescine on the in vitro regeneration potential of *L. speciosa* induced by TDZ. The highest induction of shoot buds was achieved on WPM medium supplemented with mT (5µM) + put (40 µM), exceeding the effects of BA and KIN. Additionally, IBA (0.5µM) was found to be the most effective hormone for *in vitro* rooting, resulting in an average of 4.5 roots with a mean length of 4.9cm in 86.8% of cultures. The regenerated plantlets, exhibiting well-developed roots, were successfully acclimatized and transferred to field conditions.

FUTURE SCOPE

Putrescine used in *in vitro* regeneration may be used further to develop enhanced shoot multiplication and propagation of medicinal plant.

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Conflict of Interest. None.

REFERENCES

Ahmad, A., Ahmad, N., Anis, M., Alatar, A. A., Abdel-Salam, E. M., Qahtan, A. A. and Faisal, M. (2021). Gibberellic acid and thidiazuron promote micropropagation of an endangered woody tree (*Pterocarpus marsupium* Roxb.) using in vitro seedlings. *Plant Cell, Tissue and Organ Culture (PCTOC), 144*, 449-462.

Ahmad, N. and Anis, M. (2007). Rapid clonal multiplication of a woody tree, *Vitex negundo* L. through axillary shoots proliferation. *Agroforest Syst., 71*, 195-200.

Ahmad, N. and Anis, M. (2011). An efficient in vitro process for recurrent production of cloned plants of *Vitex negundo* L. *Eur. J. For. Res*., *130*, 135-144.

Ahmad, A. and Anis, M. (2019) Meta-topolin improves in vitro morphogenesis, rhizogenesis and biochemical analysis in *Pterocarpus marsupium* Roxb: a potential drug-yielding tree. *J. Plant Growth Regul., 38*, 1007-1016.

Ahmad, N., Faisal, M., Ahmad, A., Alatar, A. A., Qahtan, A. A. and Alok, A. (2022). Thidiazuron induced in vitro clonal propagation of *Lagerstroemia speciosa* (L.) Pers.—An important avenue tree. *Horticulturae*, *8*(5), 359.

Ahmad, N. and Faisal, M. (Eds.). (2018). *Thidiazuron: From urea derivative to plant growth regulator* (pp. 1-491). Singapore: Springer.

Alam, N., Ahmad, A., Ahmad, N. and Anis, M. (2023). Polyamines Mediated In Vitro Morphogenesis in Cotyledonary Node Explants of *Mucuna pruriens* (L.) DC.: A Natural Source of L-Dopa. *Journal of Plant Growth Regulation*, 1-13.

Anwar, R., Mattoo, A. K. and Handa A. K. (2015). Polyamine interactions with plant hormones: crosstalk at several levels. *In Polyamines* (pp. 267-302). Springer, Tokyo.

Bais, H. P., Sudha, G. S. and Ravishankar, G. A. (2000). Putrescine and silver nitrate influences shoot multiplication, in vitro flowering and endogenous titers of polyamines in *Cichorium intybus* L. cv. Lucknow local. *Journal of Plant Growth Regulation*, *19*, 238-248.

Bartel, B., Leclere, S., Magidin, M. and Zolman, B. K. (2001). Inputs to the active indole-3-acetic acid pool: de novo synthesis, conjugate hydrolysis, and indole-3-butyric acid b-oxidation. *J. Plant Growth Regul., 20*, 198-216.

Bonga, J. M. and Aderkas, P. V. (1993). Rejuvenation of tissues from mature conifers and its implications for propagation in vitro. *In Clonal forestry I* (pp. 182-199). Springer, Berlin, Heidelberg.

Deepa, A. V., Anju, M. and Dennis Thomas, T. (2018). The applications of TDZ in medicinal plant tissue culture. *Thidiazuron: from urea derivative to plant growth regulator*, 297-316.

Dewir, Y. H., Naidoo, Y. and Teixeira da Silva, J. A. (2018). Thidiazuron-induced abnormalities in plant tissue cultures. *Plant cell reports*, *37*, 1451-1470.

De Klerk, G. J., Brugge, J. T. and Marinova, S. (1997). Effectiveness of indoleacetic acid, indolebutyric acid and naphthaleneacetic acid during adventitious root formation in vitro in Malus 'Jork 9'. *Plant cell tissue organ cult., 49*, 39-44.

Dey, A., Hazra, A. K., Nongdam, P., Nandy, S., Tikendra, L., Mukherjee, A. and Pandey, D. K. (2019). Enhanced bacoside content in polyamine treated in-vitro raised *Bacopa monnieri* (L.) Wettst. *South African Journal of Botany*, *123*, 259-269.

Flasiński, M. and Hąc-Wydro, K. (2014). Natural vs synthetic auxin: studies on the interactions between plant hormones and biological membrane lipids. *Environ. Res., 133*, 123-134.

Gentile, A., Jàquez Gutiérrez. M, Martinez, J., Frattarelli, A., Nota, P. and Caboni, E. (2014). Effect of meta-Topolin on micropropagation and adventitious shoot regeneration in *Prunus* rootstocks. *Plant Cell Tissue Org Cult., 118*, 373-381.

Gharari, Z., Bagheri, K., Sharafi, A. and Danafar, H. (2019). Thidiazuron induced efficient in vitro organogenesis and regeneration of *Scutellariaborn muelleri*: an important medicinal plant. *In Vitro Cellular & Developmental Biology-Plant, 55*, 133-138.

Giri, C.C., Shyamkumar, B. and Anjaneyulu, C. (2003). Progress in tissue culture, genetic transformation and applications of biotechnology to trees: an overview. *Trees 18*, 115–135.

Husain, M. K., Anis, M. (2009). Rapid in vitro multiplication of

Melia azedarach L. (a multipurpose woody tree). *Acta physiologiae plantarum*, *31*, 765-772.
Hussain, S. A., Ahmad, N., Anis, M. and Hakeem, K. R. (2019). Development of an efficient micropropagation system for *Tecoma stans* (L.) Juss. ex Kunth using thidiazuron and effects on phytochemical constitution. *In Vitro Cellular & Developmental Biology-Plant*, *55*, 442-453.
Hussain, S. S., Ali, M., Ahmad, M. and Siddique, K. H. (2011). Polyamines: natural and engineered abiotic and biotic stress tolerance in plants. *Biotechnology advances*, *29*(3), 300-311.
Huetteman, C. A. and Preece, J. E. (1993). Thidiazuron: a potent cytokinin for woody plant tissue culture. *Plant cell, tissue and organ culture*, *33*, 105-119.
Javed, S. B. and Anis, M. (2015). Cobalt induced augmentation of in vitro morphogenic potential in *Erythrina variegata* L.: a multipurpose tree legume. *Plant Cell Tissue Organ Cult., 120*, 463–474.
Jayaprakash, K., Manokari, M., Cokulraj, M., Dey, A., Faisal, M., Alatar, A. A. and Shekhawat, M. S. (2023). Improved organogenesis and micro-structural traits in micropropagated plantlets of *Caralluma umbellata* Haw. in response to Meta-Topolin. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 1-14.
Khanam, M. N., Javed, S. B., Anis, M. and Alatar, A. A. (2020). meta-Topolin induced in vitro regeneration and metabolic profiling in *Allamanda cathartica* L. *Ind Crops Prod., 145*, 111-944.
Klein, G., Kim, J., Himmeldirk, K., Cao, Y. and Chen, X. (2007). Antidiabetes and anti-obesity activity of *Lagerstroemia speciosa*. *Evid-Based Complement Alternat Med., 4*, 401-407.
Letham, D. S. and Palni, L. M. S. (1983) The Biosynthesis and Metabolism of Cytokinins. *Annu. Rev. Plant Physiol 34*, 163–197.
Lloyd, G. and McCown, B. (1980). Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture., 30, 421-427.
Magyar-Tábori, K., Dobránszki, J., Teixeira da Silva J.A., Bulley, S. M. and Hudák, I. (2010). The role of cytokinins in shoot organogenesis in apple. *Plant Cell Tissue Organ Cult., 101*, 251-267.
Manokari, M., Priyadharshini, S., Jogam, P., Dey, A. and Shekhawat, M. S. (2021). Meta-topolin and liquid medium mediated enhanced micropropagation via ex vitro rooting in *Vanilla planifolia* Jacks. ex Andrews. *Plant Cell, Tissue Organ Cult., 146*, 69-82.
Martin-Tanguy, J. (2001). Metabolism and function of polyamines in plants: recent development (new approaches). *Plant growth regulation*, *34*, 135-148.
Minocha, R., Majumdar, R. and Minocha, S. C. (2014). Polyamines and abiotic stress in plants: a complex relationship. *Front Plant Sci., 5*, 175.
Naaz, A., Hussain, S.A., Anis, M. and Alatar, A. A. (2019). Meta-topolin improved micropropagation in *Syzygium cumini* and acclimatization to ex vitro conditions. *Biol Plant, 63*, 174-182.
Nisler, J. (2018). TDZ: mode of action, use and potential in agriculture. *Thidiazuron: from urea derivative to plant growth regulator*, 37-59.
Park, W. T., Kim, Y. B., Sathasivam, R., Kim, H. H. and Park, S. U. (2022). Effect of silver nitrate and putrescine on in vitro shoot organogenesis of *Polygonum multiflorum*. *Journal of Phytology*, *14*, 36-40.
Pramanik, B., Sarkar, S., Bhattacharyya, S. and Gantait, S. (2021). meta-Topolin-induced enhanced biomass production via direct and indirect regeneration, synthetic seed production, and genetic fidelity assessment of *Bacopa monnieri* (L.) Pennell, a memory-booster plant. *Acta Physiol. Plant, 43*, 1-14.
Rashotte, A. M., Poupart, J., Waddell, C. S. and Muday, G. K. (2003). Transport of the two natural auxins, indole-3-butyric acid and indole-3-acetic acid, in Arabidopsis. *Plant Physiol., 133*, 761-772.
Scaramagli, S., Bueno, M., Torrigiani, P., Altamura, M. M., Capitani, F. and Bagni, N. (1995). Morphogenesis in cultured thin layers and pith explants of tobacco. II. Early hormone-modulated polyamine biosynthesis. *J. plant physiol., 147*, 113-117.
Shekhawat, J. K., Rai, M. K., Shekhawat, N. S. and Kataria, V. (2021). Synergism of m-topolin with auxin and cytokinin enhanced micropropagation of *Maytenus emarginata*. *In Vitro Cell. Dev. Biol., 57*, 418-426.
Shi, H. and Chan, Z. (2014). Improvement of plant abiotic stress tolerance through modulation of the polyamine pathway. *J Integr Plant Biol., 56*, 114-121.
Sivanandhan, G., Mariashibu, T. S., Arun, M., Rajesh, M., Kasthurirengan, S., Selvaraj, N. and Ganapathi, A. (2011). The effect of polyamines on the efficiency of multiplication and rooting of *Withania somnifera* (L.) Dunal and content of some withanolides in obtained plants. *Acta Physiologiae Plantarum*, *33*, 2279-2288.
Tavladoraki, P., Cona, A., Federico, R., Tempera, G., Viceconte, N., Saccoccio, S. and Agostinelli, E. (2012). Polyamine catabolism: target for anti proliferative therapies in animals and stress tolerance strategies in plants. *Amino acids*, *42*, 411-426.
Unno, T., Sakan, I., Masumizu, T., Kohno, M. and Kakuda, T. (1997). Antioxidative activity of water extracts of *Lagerstroemia speciosa* leaves. *Biosci. Biotechnol. Biochem., 61*, 1772-1774.
Vasudevan, V., Subramanyam, K., Elayaraja, D., Karthik, S., Vasudevan, A. and Manickavasagam, M. (2017). Assessment of the efficacy of amino acids and polyamines on regeneration of watermelon (*Citrullus lanatus* Thunb.) and analysis of genetic fidelity of regenerated plants by SCoT and RAPD markers. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *130*, 681-687.
Vanisree, M., Lee, C. Y., Lo, S. F., Nalawade, S. M., Lin, C. Y. and Tsay, H. S. (2004). Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures. *Bot. Bull. Acad. Sin*, 45, 1-22
Vengadesan, G., Ganapathi, A., Anand, R. P. and Selvaraj, N. (2003). In vitro propagation of *Acacia sinuata* (Lour.) Merr. from nodal segments of a 10-year-old tree. *In Vitro Cellular & Developmental Biology-Plant*, *39*, 409-414.
Vijayan, A., Padmesh Pillai P., Hemanthakumar, A. S. and Krishnan, P. N. (2015). Improved in vitro propagation, genetic stability and analysis of corosolic acid synthesis in regenerants of *Lagerstroemia speciosa* (L.) Pers. by HPLC and gene expression profiles. *Plant Cell Tissue and Organ., 120*, 1209–1214.
Werbrouck, S. P. O., Strnad, M., Van Onckelen, H. A. and Debergh, P. C. (1996). Meta-topolin, an alternative to benzyladenine in tissue culture?*Physiol Plant, 98*, 291–297.
Yamada, K., Hosokawa, M., Yamada, C., Watanabe, R., Fujimoto, S., Fujiwara, H. and Inagaki, N. (2008). Dietary Corosolic acid ameliorates obesity and hepatic steatosis in KK-Ay mice. *Biol. Pharm. Bull., 31*, 651-655.
Zolman, B. K., Yoder, A. and Bartel, B. (2000). Genetic analysis of indole-3-butyric acid responses in Arabidopsis thaliana reveals four mutant classes. *Genetics, 156*, 1323-1337.

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