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Research article

Effect of various plant growth regulators on *in vitro* seed germination and shoot organogenesis in *Tectona grandis* L.f.

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Abstract: In the present study efforts were made to enhance *in vitro* seed germination and achieve shoot organogenesis through different explants of *Tectona grandis*, a hardwood timber species known throughout the world for its high-value wood. Seed germination was significantly affected by seed inoculation on different strengths of MS medium and different GA_3 concentrations. Different strengths of MS media combined with different GA_3 concentration significantly affect seed germination *in vitro*. Maximum seed germination (96%) was obtained on 0.4% GA_3 combined with half strength MS medium. The effect of types of explants (leaf, internode, hypocotyl and root of seedlings), different concentrations of thidiazuron (TDZ) (0.1, 1.0 and 10 µM) and auxins (IBA, IAA and NAA) on callus induction and shoot formation was investigated. After 30 days of inoculation, it was observed that explant type had a significant effect on callus formation. Maximum callus was obtained on internode explants (56.17%) which were statistically on par with callus obtained on leaf explants (54.32%). The effect of TDZ on callus and shoot formation was also observed. TDZ with higher concentration (1 µM) gave maximum callus (48.6%). Whereas lower concentration of TDZ (0.1 µM) was effective for shoot formation from both internode and hypocotyl explants. IAA and NAA were also helpful in shoot formation whereas IBA retards shoot formation. For shoot organogenesis internode was found to be most responsive explant type followed by hypocotyls and leaf. Roots were found to be the least responsive explant.

Keywords: Auxins - Callus - Explant - Gibberellic acid - Shoot organogenesis.

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INTRODUCTION

Tectona grandis L.f. (Teak) is distributed naturally in many countries of South-East Asia. It is a paragon amongst the hardwood timber species. It has been planted in a number of tropical countries because of its amenability for plantations coupled with desirable wood properties and durability. India is considered to be the center of diversity for teak and it is widely distributed in central and south India, occupying about 13% of the total forest area (Tewari 1992). The Indian region is considered to be the center of genetic diversity of teak (Anonymous 2006). *Tectona grandis* is found in a variety of habitats and climatic conditions from arid areas with only 500 mm of rain per year to very moist forests with up to 5000 mm of rain per year. In teak growing areas, average annual rainfall is usually 1250–1650 mm with a 3–5 months dry season It occurs in natural forests between 9° to 26° N latitude and 73° to 104° E longitude, which includes southern and central India, Myanmar, Laos People's Democratic Republic and northern Thailand (White 1991).

Teak is generally propagated from seeds. Various methods of vegetative propagation have been used, such as budding and grafting (Mahmood & Somasundaram 1975), rooting of cuttings (Lahiri 1974) and rooting of buds cut from stock stumps raised in polypots (Mahmood & Somasundaram 1975). The conventional methods of vegetative propagation have limitations. They are slow and time-consuming. Teak being a cross-pollinated

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species, progeny raised from seed show wide variation. It also has an irregular seed-bearing habit and the production of seed tends to be much lower than the requirement (Lee & Rao 1981). The viability of seed is poor and is affected by the season when the seed is collected and by the storage conditions (Department of Forestry. New Guinea 1962). The seeds are enclosed in hard coats and show very poor germination rates in spite of various pretreatments (Joshi & Kelkar 1971, Dabral & Amin 1975, Dabral 1976, Davidson & Fairlamb 1976). Mathew & Vasudeva (2003) also reported low seed germination in seeds collected from clonal seed orchards. These characteristics have become a major problem in large-scale planting. In the face of the rising demand for teak planting stock, the traditional tree improvement and multiplication programmes are not productive enough. A rapid means of producing a large number of plants for afforestation is offered by micropropagation Gupta *et al.* (1980) estimated that though subculture, 3000 viable plants from a single seedling or 500 plants can be obtained from a single bud of a mature plant in one year.

Genetic improvement of teak by conventional breeding methods is still difficult due to the long reproductive cycle. It takes more than a decade from the initiation of a genetic improvement program until improved seeds are available and need another 4–5 decades until the timber from the first rotation of improved planting stock can be harvested. Therefore, special attention must be given for producing genetically engineered teak to by-pass the long period required for natural genetic crosses and selection (Widiyanto *et al*. 2009). However, no report of the successful genetic transformation of teak has been published to date (Widiyanto *et al*. 2009). In attempts to develop the transformation procedure of teak, many researchers have studied the regeneration ability of teak callus tissues to develop shoots and found that the frequency of adventitious shoot formation from teak callus was either very low or absent (Widiyanto *et al*. 2003, Widiyanto *et al*. 2005). Therefore, in the present study, two experiments were conducted. Firstly attempts were made to study the effect of GA_3 treatments and MS medium strengths on seed germination. The second experiment was conducted to investigate the effect of thidiazuron (TDZ), auxins and explant types on adventitious shoot organogenesis in teak.

MATERIALS AND METHODS

Collection and preparation of seeds

Dried teak fruits were collected from phenotypically superior trees of *Tectona grandis* present in Tropical Forest Research Institute, Jabalpur. The fruits were opened with a nutcracker. The seeds were thoroughly washed with distilled water and then immersed and agitated in 0.1 % Cetrimide solution for 20 min on a Rotary shaker. Thereafter, the seeds were washed with distilled water 3–4 times and treated with 0.1% streptomycin and 0.2% Bavistin® solution for 10 min and again rinsed with distilled water 3–4 times. Next, in the laminar airflow, the seeds were treated with 0.1 % mercuric chloride solution for 5 min and rinsed thoroughly three times with sterile distilled water to completely wash off the compound.

Germination of seed

The surface-sterilized seeds were inoculated individually in semi-solid MS medium. In order to optimize the germination, the seeds were inoculated on three strengths of MS medium supplemented with four concentrations of GA_3. A two way factorial randomised experiment was conducted to study the effect of different strengths of MS medium (Full, ½ and ¼ strength of MS salts), different concentrations of GA_3 (0, 0.1 %, 0.2 % and 0.4%) and their interactions on germination (%) in the seeds of teak. The experiment consisted of 12 treatments and three replicates. There were 10 explants per treatment. The observations for germination percentage were recorded 10, 15 and 30 days after seed inoculation.

Callus formation

After germination, the *in vitro* raised seedlings produced 2–3 nodes in 20–25 days. The different parts of these seedlings were used for callus and shoot formation. A two way factorial randomised experiment was designed to study the effect of different strengths of TDZ (0.1, 1 and 10 µM), different auxins (0.1 µM) (IBA, IAA and NAA) and their interactions on callus formation/organogenesis in the different explants obtained from the seedling *viz.*, internode, leaves, roots and hypocotyl. The experiment consisted of 9 treatments and three replicates. There were 10 explants per treatment and data was recorded at an interval of 15 and 30 days.

Culture conditions

After inoculation, the test tubes containing seeds were incubated in dark conditions for 7 days to induce germination. After germination, the cultures were transferred in light condition under a controlled set of environmental conditions in the culture room. They were incubated at 25±2C with 16 hr photoperiod provided by cool white fluorescent tubes of 40 watts (approx. 45 µmol m^{-2} s^{-1}). The inorganic salts used for the

preparation of culture medium were obtained from SRL Chemicals and Qualigens Pvt. Ltd., India and phytohormones and B vitamins from Sigma Chemicals Pvt. Ltd., India. The medium contained 3% (w/v) sucrose as carbon source and 0.8% (w/v) agar (Microexpress Pvt. Ltd., India). The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl before autoclaving for 15 min at 1.06 kg cm^{-2} (121°C). Each seed was cultured in a 2.5 cm × 15.0 cm glass test tube (Borosil India Ltd.) containing 10 ml sterilized semi-solid medium. for culture initiation and 400 ml culture bottles containing 45 ml semi-solid medium for organogenesis.

Statistical analysis

The seed germination rate was expressed as a percentage, which was calculated by using the following equation: (number of germinated seeds/total number of seeds inoculated per treatment) X 100. All experiments were conducted in complete randomized design (CRD) and data were analyzed by two-way analysis of variance using SX statistical package. The significance of the data was ascertained by F-test and the Critical Difference (C.D.) values at p = 0.05 computed for comparing means of various treatments. The factorial combination was used to study the interactions of the treatments. Arc sine transformation was used to transform data expressed in percentage (Gomez & Gomez 1984).

RESULTS

Seed Germination

Effect of GA_3 treatment: Germination of seeds started one week after inoculation of seeds. The germination percentage of seeds after 10, 15 and 30 days of inoculation was significantly affected by different concentrations of GA_3 (Table 1). The seed germination obtained on 0.1%, 0.2% and 0.4% GA_3 treatments was 42.59%, 42.58% and 38.88%, respectively. After 15 days of seed inoculation, germination increased to 57.40% on 0.1% GA_3, 49.99% on 0.2% and 49.99% on 0.4% GA_3 treatment, respectively. After 30 days of inoculation, maximum seed germination (96.29%) was obtained on treatment of 0.4% GA_3, which was statistically on par with germination obtained on 0.2% GA_3 treatment (92.59%) (Fig. 1A–D). Even after one month, seed germination obtained on control (treatment without GA_3 treatment) was very low (9.25%).

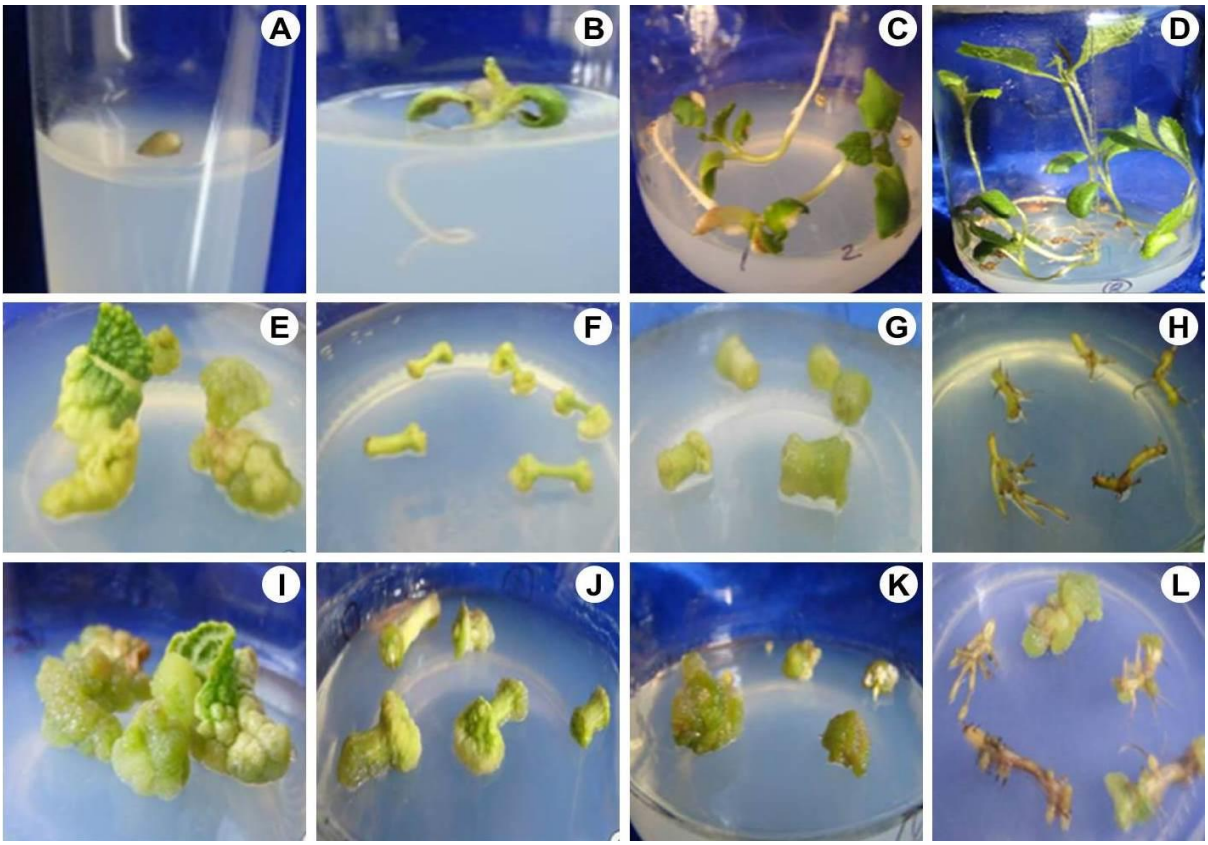

Figure 1. *In vitro* seed germination in seeds of *Tectona grandis*: **A**, Seed inoculation; **B**, Germination after 10 days; **C**, Germination after 15 days; **D**, Germination after 30 days; **E–L**, Callus formation in leaf, internodes, hypocotyls and root on MS medium supplemented with 1 µM TDZ & 0.1 µM IBA (**E**), 0.1 µM TDZ & 0.1 µM NAA (**F**), 1 µM TDZ & 0.1 µM IBA (**G**), 10 µM TDZ & 0.1 µM IAA (**H**) after 15 days of inoculation and 10 µM TDZ & 0.1 µM IBA (**I**), 1 µM TDZ & 0.1 µM NAA (**J**), 1 µM TDZ & 0.1 µM IBA (**K**), 10 µM TDZ & 0.1 µM IAA (**L**) after 30 days of inoculation.

Effect of MS medium strengths: Highly significant effect of MS medium strengths was observed on germination of seeds after 30 days of inoculation. Maximum germination of seeds (79.16%) was obtained on 1/2 strength MS medium which was significantly higher than seed germination obtained on any other MS medium strength. Least germination of seeds (61.10%) was obtained on full strength MS medium.

Table 1. Effect of different strengths of MS medium, GA_3 and their interactions on seed germination in *Tectona grandis* after 10, 15 and 30 days of inoculation. (The data expressed in parenthesis are arc sine transformed)

GA_3 (%) (G)	MS strength (MS) 10 days Full	Half	Quarter	Mean	MS strength (MS) 15 days Full	Half	Quarter	Mean	MS strength (MS) 30 days Full	Half	Quarter	Mean
0	0	0	11.10	3.70	0	16.66	11.10	9.25	0	16.66	11.10	9.25
	(0.04)	(0.04)	(16.04)	(5.37)	(0.04)	(19.77)	(16.04)	(11.95)	(0.04)	(19.77)	(16.04)	(11.95)
0.1	49.99	44.44	33.33	42.59	72.21	55.55	44.44	57.40	72.21	100	77.77	83.33
	(44.98)	(41.75)	(35.24)	(40.66)	(58.43)	(48.23)	(41.75)	(49.47)	(58.43)	(89.94)	(62.15)	(70.17)
0.2	44.44	49.99	33.33	42.58	49.99	61.11	38.88	49.99	83.33	100	94.44	92.59
	(41.73)	(44.98)	(34.76)	(40.49)	(44.98)	(51.96)	(38.49)	(45.14)	(71.18)	(89.96)	(81.93)	(81.02)
0.4	49.99	33.33	33.33	38.88	49.99	49.99	49.99	49.99	88.88	100	100	96.29
	(44.98)	(34.76)	(34.76)	(38.17)	(44.98)	(45.45)	(44.98)	(45.14)	(73.91)	(89.96)	(89.96)	(84.61)
Mean	36.10	31.94	27.77		43.04	45.82	36.10		61.10	79.16	70.83	
	(32.93)	(30.38)	(30.20)		(37.11)	(41.35)	(35.31)		(50.89)	(72.41)	(62.52)	

Variable	$CD_{(0.05)}$ 10 days	15 days	30 days
G	8.92	10.96	10.57
MS	N.S.	N.S.	9.61
G*MS	N.S.	N.S.	N.S

Adventitious shoot organogenesis

The objective of this experiment was to investigate the effect of types of explant (leaf, internode, hypocotyl and root of seedling), different concentrations of TDZ (0.1, 1.0 and 10 µM) and auxins (IBA, IAA and NAA) in MS medium on callus induction and shoot formation.

Callus formation

Leaf curling and elongation of leaves started after 7–8 days of inoculation on media. The swelling was observed on the surface of internodes after 7–8 days of inoculation. Callus induction was initiated on the leaf pieces, internodes and hypocotyls after 10–12 days of inoculation. Colour of the root explants changed to green from brown after 7–8 days and the length of roots increased. Callus formation on the root explants started after 15 days of inoculation. The data for callus formation was recorded after 15 and 30 days of inoculation (Fig. 1E–L).

Effect of explants type and auxins

Table 2. Effect of explant type, auxins and their interactions on callus induction in *Tectona grandis* after 15 and 30 days of inoculation. The data expressed in parenthesis are arc sine transformed.

Explant type (E)	Auxins (0.1 µM)(A) 15 days IBA	IAA	NAA	Mean	30 days IBA	IAA	NAA	Mean
Leaf	14.81	0.00	14.81	9.87	48.15	55.55	59.26	54.32
	(16.95)	(0.04)	(17.11)	(11.36)	(45.49)	(49.82)	(50.71)	(48.67)
Internode	22.22	25.92	25.92	24.69	59.26	55.55	53.70	56.17
	(23.03)	(25.35)	(27.09)	(25.15)	(54.05)	(49.98)	(50.48)	(51.51)
Hypocotyl	22.22	18.52	9.25	16.66	40.74	44.44	27.77	37.65
	(24.77)	(20.86)	(13.37)	(19.67)	(39.41)	(39.98)	(28.17)	(35.85)
Root	9.25	7.40	0.00	5.55	24.07	9.25	1.85	11.73
	(10.53)	(9.28)	(0.04)	(6.61)	(22.52)	(10.53)	(2.70)	(11.92)
Mean	17.13	12.96	12.50		43.05	41.20	35.65	
	(18.82)	(13.88)	(14.40)		(40.37)	(37.58)	(33.02)	

Variable	$CD_{(0.05)}$ 15 days	30 days
E	8.95	15.25
A	7.75	13.21
E*A	15.46	26.42

It was observed that explant type had a significant effect on callus induction after 15 days of inoculation. Maximum callus was formed on the surface and cut ends of internodes (24.69%) which were statistically on par with callus formed on hypocotyls (16.66%). Least callus induction was obtained on root explant. After 30 days of inoculation, it was observed that explant type had a significant effect on callus formation (Table 2). Maximum callus was obtained on internode explants (56.17%) which were statistically on par with callus obtained on leaf explant (54.32%). The effect of auxins and interaction between explant type and auxins was found to be statistically not significant for callus induction after 15 and 30 days of inoculation.

Effect of explant type and thidiazuron (TDZ)

The effect of TDZ on callus formation was also observed to be significant after 30 days of inoculation (Table 3). Maximum callus was obtained on 1 µM TDZ (48.67%) which was statistically on par with callus obtained on 10 µM TDZ (40.74 %).

Table 3. Effect of explant type, thidiazuron (TDZ) and their interactions on callus induction in *Tectona grandis* after 15 and 30 days of inoculation. The data expressed in parenthesis are arc sine transformed.

Explant type (E)	TDZ (µM) (T)							
	15 days			Mean	30 days			Mean
	0.1	1	10		0.1	1	10	
Leaf	5.55 (6.61)	14.81 (15.52)	9.25 (11.95)	9.87 (11.36)	38.89 (36.58)	61.11 (53.39)	62.96 (56.06)	54.32 (48.67)
Internode	37.03 (35.50)	24.07 (24.26)	12.96 (15.70)	24.69 (25.15)	50.00 (48.16)	70.37 (62.29)	48.15 (44.07)	56.17 (51.51)
Hypocotyl	14.81 (16.95)	24.07 (27.44)	11.11 (14.62)	16.66 (19.67)	14.81 (18.53)	53.70 (47.30)	44.44 (41.74)	37.65 (35.85)
Root	3.70 (3.95)	9.25 (11.95)	3.70 (3.95)	5.55 (6.61)	18.52 (16.10)	9.25 (10.37)	7.40 (9.28)	11.73 (11.92)
Mean	15.28 (15.75)	18.05 (19.79)	9.25 (11.56)		30.55 (29.84)	48.61 (43.34)	40.74 (37.79)	

Variable	$CD_{(0.05)}$	
	15 days	30 days
E	8.95	15.25
T	7.75	13.21
E*T	15.46	26.42

Shoot formation

Shoot primordia were visible after 30 days of inoculation. For shoot organogenesis hypocotyl was found to be most responsive explant type followed by internode. The lowest concentration of TDZ (0.1 µM) was effective for shoot formation on internode explants of teak (Fig. 2A–B). Shoot formation was obtained on treatments of IAA and NAA but not on IBA.

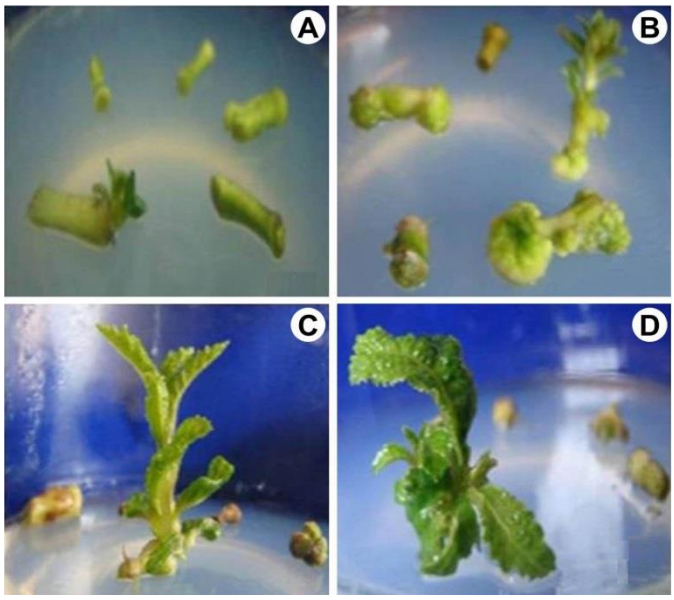

Figure 2. Shoot formation after 30 days of inoculation: **A–B**, Internode explants on MS medium supplemented with 0.1 µM TDZ & 0.1 µM IAA (**A**) and 0.1 µM TDZ & 0.1 µM IBA (**B**); **C–D**, Hypocotyl explants on MS medium supplemented with 0.1 µM TDZ and 0.1 µM IAA (**C**) and 0.1 µM TDZ and 0.1 µM NAA (**D**).

With hypocotyl explants also, lower concentrations of TDZ (0.1 and 1 µM) were more helpful for shoot formation than higher concentration (10 µM) (Table 4) (Fig. 2C–D). Shoot formation was obtained with all three auxins (IAA, IBA and NAA).

Table 4. Effect of thidiazuron (TDZ), different auxins and their interactions on number of shoots formed on internode in *Tectona grandis* after 30 days inoculation.

Auxins (0.1µM)(A)	TDZ (µM) (T)							
	Internode			Mean	Hypocotyl			Mean
	0.1	1	10		0.1	1	10	
IBA	0.00	0.00	0.00	0.00	0.26	0.10	0.00	0.12
IAA	0.06	0.00	0.00	0.02	0.20	0.20	0.10	0.16
NAA	0.13	0.00	0.00	0.04	0.06	0.06	0.01	0.04
Mean	0.06	0.00	0.00		0.17	0.12	0.03	

Variable	$CD_{(0.05)}$	
	Internode	Hypocotyl
A	0.08	0.20
T	0.08	0.20
A*T	0.12	0.35

DISCUSSION

The increasing demand of teak for plantation purposes by forest departments as well as private companies has necessitated research on unconventional methods for the improvement of productivity. The potential benefits of the use of clonal planting stock in reforestation programs have long been recognized. However, to achieve the maximum possible genetic gain for teak improvement, both sexual reproduction and vegetative multiplication must be followed. This can be accomplished through micropropagation using seeds as explants by germinating them under *in vitro* conditions.

In vitro propagation has been successfully applied in *Tectona grandis* and it has become an alternative tool to overcome some problems occurring in sexual regeneration (Widiyanto *et al.* 2005). Regenerative organs such as pre-existing shoots, meristem shoot-tips, nodal segments or seedling organs have been widely used as explants (Gupta *et al.* 1980, Mascarenhas *et al.* 1987, Apavatjrut *et al.* 1988, Devi *et al.* 1994). Adventitious shoot formation from callus tissues has been proposed for the regeneration of genetically engineered tissues of many species (Siemens & Schieder 1996, Tawfik & Noga 2001, Lee & Pijut 2017).

Gibberellic acid is used in laboratory and greenhouse conditions to trigger germination in seeds that would otherwise remain dormant. It is a naturally occurring plant growth regulator which may cause a variety of effects including the stimulation of seed germination in some cases. GA_3 occurs naturally in the seeds of many species and is produced commercially by growing *Gibberella fujikuroi* (Sawada) Wollenw. fungus cultures in vats, then extracting and purifying the GA_3 (Yamaguchi 2008, Santner *et al.* 2009). The GA_3 treatments accelerated the process of seed germination and germination started early in treated seeds as compared to control. It stimulates the synthesis and production of the hydrolases, especially amylase, resulting in the germination of seeds. Presoaking seeds in GA_3 solution will in many cases cause the rapid germination of many types of highly dormant seeds which would otherwise need cold treatment, after-ripening or ageing, or other prolonged pretreatments. Similar to our results Ribeiro *et al.* (2009) reported a positive effect of GA_3 on seed germination in *Annona crassiflora* Mart. Similarly, in *Juglans regia* L. also enhanced seed germination was obtained (Kaur *et al.* 2006).

Generally, media high in salt and sugar content reduced germination efficacy. Maximum germination was obtained on the lower strength of MS media (1/2). Least germination of seeds (61.10%) was obtained on full strength MS medium. These results are in agreement with Ranasinqhe & Berlyn (1996) who reported only 50% seed germination of teak on full strength MS medium as opposed to 60% on half strength. This effect could partly be attributed to the role minerals play as osmotica. Because any germination process is preceded by imbibition, anything that affects this process is likely to affect germination.

Adventitious shoot organogenesis

The induction of callus growth and organogenesis or differentiation of shoot buds directly or indirectly from explants, is accomplished by the differential application of growth regulators and control of physical conditions. In teak only a very few reports are there for adventitious organogenesis.

Callus formation

The type of explant used had a significant effect on callus formation (%). Maximum callus was obtained on

internode explant which was statistically on par with hypocotyl explant. But the effect of auxins, TDZ and their interactions was found to be statistically non-significant for callus formation. Similarly, Kozgar & Shahzad (2012) also did not achieve any enhanced results on combination treatment of BA, kinetin and TDZ in teak.

Shoot formation

Internode and hypocotyls explants of teak proved to be the most responsive explant type among the four explants tried. Widiyanto *et al*. (2005) also reported shoot organogenesis in teak on calli induced from internodal segments on Woody Plant Medium containing 1.0 µM thidiazuron (TDZ) in combination with 0.01 µM indole butyric acid (IBA).

CONCLUSION

The present investigation concentrated on finding out the most suitable GA_3 concentration and MS media strength for *in vitro* seed germination of *Tectona grandis* on one hand and on the other hand to screen out the most suitable explant type and plant growth regulator type and concentration for shoot organogenesis. Looking at the results of the present study pre-treatment of teak seeds with 0.4 % GA_3 and germination on 1/2 strength MS medium can be recommended. For shoot organogenesis internode was found to be most responsive explant type followed by hypocotyls and leaf. Roots were found to be the least responsive explant.

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REFRENCES

Anonymous (2006) *The Wealth of India, Second Supplement Series on raw materials, Volume 3*. CSIR, New Delhi.

Apavatjrut P, Kaosa-Ard A & Paratasiplin T (1988) Current research on teak (*Tectona grandis* Linn. f.) tissue culture in Thailand. *BIOTROP Special Publication* 35: 107–115.

Dabral SL & Amin PW (1975) Poor fruit formation in teak in Chanda Forests of Maharashtra. *Indian Forester* 101: 616–619.

Dabral SL (1976) Extraction of teak seeds from emits, their storage and germination. *Indian Forester* 102: 650–658.

Davidson J & Fairlamb J (1976) Germination of teak seeds- Preliminorv evidence of a chemical germination inhibitor. In: *Seed problems*. Proceedings of the 2nd International IUFRO Symposium on Physiology of Seed Germination, Japan.

Devi YS, Mukherjee BB & Gupta S (1994) Rapid cloning of elite teak (*Tectona grandis* Linn.) by *in vitro* multiple shoot production. *Indian Journal of Experimental Biology* 32: 668–671.

Gomez K A & Gomez AA (1984) *Statistical Procedures for Agricultural Research.* John Wiley and Sons, Inc., New York, 680 p.

Gupta PK, Nadgir AL, Mascarenhas A & Jagannathan V (1980) Tissue culture of forest trees: clonal multiplication of *Tectono grandis* (teak) by tissue culture. *Plant Science Letters* 17: 259–268.

Joshi MD & Kelkar SP (1971) Germination of seed in dry teak *(Tectono grandisi* (I) Preliminary studies in fruit development and seed dormancy. *Indian Forester* 97: 210–212.

Kaur R, Sharma N, Kumar K, Sharma DR & Sharma SD (2006) *In vitro* germination of walnut (*Juglans regia* L.) embryos. *Scientia Horticulturae* 109: 385–388.

Kozgar MI & Shahzad A (2012) An improved protocol for micropropagation of teak tree (*Tectona grandis* L.). *Rendiconti Fisiche Accademia Lincei* 23: 195–202.

Lahiri AK (1974) Preliminary study on rooting of green wood cutting of teak. *Indian Forester* 100: 559–560.

Lee JH & Pijut PM (2017) Adventitious shoot regeneration from in vitro leaf explants of *Fraxinus nigra*. *Plant Cell Tissue and Organ Culture* 130: 335–343.

Lee SK & Rao AN (1981) *In vitro* plantlet development in tropical trees - *Callophyllum inophyllum* and *Eugenia grandis*. In: Rao AN (eds) *Tissue culture of economically important plants*.. Singapore, COSTED, pp. 185–190.

Mahmood Hussain AM & Somasundaram TR (1975) Scions on stumps - an innovation in the vegetative propagation of teak *(Tectono grandis).* South Forest Rangers College Magazine 51: 1–8.

Mascarenhas AF, Kendurkar SV, Gupta PK, Khuspe SS & Agrawal DC (1987) Teak. In: *Cell and Tissue Culture in Forestry, Volume 3*. Martinus Nijhoff Publication, Dordrecht, Boston, Lancaster, pp. 300–315.

Mathew J & Vasudeva J (2003) Clonal variation for seed germination in teak (*Tectona grandis* Linn. f.) *Current Science* 84(8): 1133–1136.

Joshi MD & Kelkar SP (1971) Germination of seed in dry teak (*Tectono grandis* L) I.Preliminary studies in fruit development and seed dormancy. *Indian Forester* 97: 210–212.

Ranasinqhe DMSHK and Berlyn GM (1996) Vegetative propagation of teak by tissue culture. Proceedings of the Second Annual Forestry Symposium 1996. In: Amarasekera HS, Ranasinghe DMSHK & Finlayson W (eds) *Management and Sustainable Utilization of Forest Resources*. Department of Forestry and Environmental Science, University of Sri Jayewardenepura, Sri Lanka.

Ribeiro MNO, Pasqual M, Villa F, Pio LAS & Hilhorst HWM (2009) *In vitro* seed germination and seedling development of *Annona crassiflora* Mart. *Science Agriculture (Piracicaba, Brazil)* 66(3): 410–413.

Santner A, Calderon-Villalobos L & Estelle M (2009) Plant hormones are versatile chemical regulators of plant growth. *Nature Chemical Biology* 5: 301–307.

Siemens J & Schieder O (1996) Transgenic plants: genetic transformation - recent developments and the state of the art. *Plant Tissue Culture and Biotechnology* 2: 66–75.

Tawfik AA & Noga G (2001) Adventitious shoot proliferation from hypocotyl and internodal stem explants of cumin. *Plant Cell Tissue and Organ Culture* 66: 141–147.

Tewari DN (1992) *A monograph on teak (Tectona grandis)*. International Book Distributors, Dehradun.

White KJ (1991) *Teak: some aspects of research and development*. FAO Regional Office for Asia and Pacific (RAFA), Bangkok, pp. 82.

Widiyanto SN, Erytrina D & Rahmania H (2005) Adventitious Shoot Formation on Teak (*Tectona grandis* L.F.) Callus Cultures Derived from Internodal Segments. *Acta Horticulture* 692: 153–155.

Widiyanto SN, Rahmania H & Suhandono S (2003) Shoot formation on *Agrobacterium* co-cultivated tissues of teak. *In Vitro Cellular and Developmental Biology* 39: 23A.

Widiyanto SN, Sukmawan A, Haro N & Rahmania H (2009) Transient expression of b-glucuronidase reporter gene in *Agrobacterium*-inoculated shoots of various teak clones. *African Journal of Biotechnology* 8(10): 2143–2150.

Yamaguchi S (2008) Gibberellin metabolism and its regulation. *Annual Review of Plant Biology* 59: 225–251.