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Comparison of morphogenic and plant regeneration ability of some explants of teak (*Tectona grandis* Linn. F)

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To search out the explants with higher regeneration potential-mature cotyledons, cotyledonary nodes and epicotyls of teak were cultured on MS basal medium with different types of auxins and cytokinins in varying concentrations and combinations. Mature cotyledon explants exhibited higher *in vitro* morphogenesis closely followed by cotyledonary node and epicotyls cultured on medium fortified with 4.0 $mg.l^{-1}$ BA and 2.0 $mg.l^{-1}$ NAA. However, maximum plantlets were recovered from cotyledonary nodes on same medium. Phenotypically normal plants were obtained after hardening in green house.

Key words: *Tectona grandis,* mature cotyledon, cotyledonary node, epicotyls, callus, *in vitro*, morphogenesis

Introduction

Teak (*Tectona grandis* Linn. F.) is an economically important, large deciduous, and semi evergreen tree. Teak enjoys a worldwide reputation as paragon among the most prized tropical timbers and is predominantly distributed in tropical and sub-tropical regions of Southeast Asia including India. Its extraordinarily wood working qualities and durability make it ideal for variety of purposes such as deckhouses, bridge building, rails, latches, weather doors, and other construction in contact with water such as docks, quays, piers and floodgates. Teak is a slow growing tree with low germination rate (usually less than 50%) that make difficult to propagate naturally and does not meet the demand of timber at present. The new gears of biotechnology will to realm us to the production of disease free true-to-type planting material and tailor teak, especially by transferring transgene having higher growth rate.

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Although, all plant cells contain identical information, callus derived from somatic cells varies in competence to express totipotency (i.e. their genetic ability to produce plants). Mostly, explants that contain meristematic cells develop callus, which is proficient to express totipotency. In teak, diverse explants have been used proficiently to produce regenerable cultures via *in vitro* morphogenesis are auxiliary buds (Kushaokar and Sharon, 1996; Ramesh *et al*., 2003), shoot buds (Chalupa, 1987), axillary buds from nodal sections (Tiwari *et al*., 2002; Ramesh *et al*., 2003), leaf discs (Ranasinghe and Beriyn, 1996; Muminova *et al*., 1999), apical buds (Kushaokar and Sharon, 1996) and axillary buds from stem segments (Nutiyal *et al*., 1992; Ranasinghe and Beriyn, 1996). Tissue culture on teak for micropropagation shows promising results with shoot tips and nodal segments. However, no protocols are available for *in vitro* morphogenesis, which is a prerequisite for successful transformation. During the present investigation an effort has been to select the best *in vitro* responsive explant for *in vitro* morphogenesis especially somatic embryogenesis. In addition, proper cytokinin-to-auxin ratio exhibiting higher *in vitro* morphogenesis frequency was determined.

Materials and methods

Experiments were conducted with different accessions of teak collected from diverse site such as Tropical Forest Research Institute, Jabalpur MP, State Forest Research Institute, Jabalpur and J.N. Agricultural University, Jabalpur. The absolute mature seed with yellowish brown hard fruit coat were collected for excision of explants viz, mature cotyledon, cotyledonary node and epicotyls.

To begin with, a preliminary experiment, two different fortifications of basal media viz, MS (Murashige and Skoog, 1962) and B_5 (Gamborg, 1968) were tested to find out better *in vitro* response. During the course of preliminary investigations, MS basal medium was found more responsive than B_5 medium (data not presented). Subsequently for later experimentations basal MS medium was used. Culture media combinations were short listed on the basis of work conducted by various scientists as well as on the basis of preliminary experiments conducted in this laboratory (data not presented). The basal MS media supplemented with different concentrations of plant growth regulators in various combinations have been given in Tables 1-4. All the initial culture media were formulated with 30.0 g L^{-1} sucrose and 7.5 g L^{-1} agar and autoclaved at 121^0C under 1.1 kg cm^{-2} for 20 min after adjusting the pH to 5.6 ± 0.1 with 1 N KOH. Readymade MS and B_5 medium were procured from Hi-media Laboratories, Mumbai, India and growth regulators from Sigma, US. Seeds of teak were thoroughly washed under running tap water for five minutes before surface sterilizing with 70 % (v/v) ethanol for 1 min followed

by a treatment of 0.1 % (w/v) $HgCl_2$ for 3 min and finally 4-5 times rinsing with sterilized water. Surface sterilized seed were inoculated in culture tubes containing agar gelled water (7.5 g L^{-1} agar) under diffused luminance of 16µ mol m^{-2} s^{-1} provided with PAR for seven days. All the explants (mature cotyledons, cotyledonary nodes and epicotyls) were obtained from 7 days old germinated seeds. Explants were plated in 100 x 17 mm glass Petridishes, 5-7 pieces of each of, sealed with Parafilm® and incubated under complete darkness at 25 ± 2ºC for 1 week. Later the cultured petridishes were subjected to 12 h photoperiod regime of 30 µmol m-^{2} s^{-1} luminance provided with white PAR lamps.

For plantlet regeneration, calli acquired from 4-5 week old cultures were placed onto fortified solid MS regeneration medium supplemented with different concentrations and combinations of plant growth regulators (BAP, Kn and NAA alone as well as BAP and Kn in combination with NAA), 20.0 g L^{-1} sucrose and 7.5 g L^{-1} agar (Table 5). Cultured baby food bottles were subjected to 25±2^{o}C temperature and photoperiod regimes of 60 mol $m^{-2}s^{-1}$ luminance provided by PAR lamps for 16 h. Frequency of plantlet regeneration was calculated as percentage of calli with plantlets from total calli plated. The regenerated plantlets were transferred to test tubes containing MS rooting medium supplemented with 1.0 mg L^{-1} IBA and 15g L^{-1} sucrose. Rooted plants thoroughly washed under running tap water to remove the adhering agar were planted in 2.5 cm root trainers filled with sterilized 1:1:1 sand, soil and FYM mixture. Transplanted plants were subjected to 30 ± 2^{o}C and 60 ± 5^{o}C RH for 3-4 weeks in a glass house for hardening before transferring to the field.

Results and discussions

For teak micropropagation, most of the efforts have been made with auxiliary buds, shoot buds, nodal sections, apical buds and stem cuttings. Furthermore, hypocotyls, epicotyls and cotyledons obtained from germinating seeds or seedlings developed under laboratory conditions may be used for plant regeneration since growth conditions of donor plants affect the *in vitro* plant regeneration efficacy up to a great extent.

All the cultured explants enlarged during initial 7-8 days and no callus proliferation was observed. During this period explants responded in a similar manner mostly independent from culture media and conditions. During second week of culture, callus initiated from the edges of mature cotyledons and cotyledonary nodes (Fig. 1A). Where as from epicotyl segments profuse callus growth started from the cut ends. The calli from various explants generated on different media combinations varied in texture and colour. Discrete phenotypes

of proliferated calli were observed viz. wet, rough, fragile, dense and glossy in texture and white, dark/ light green and yellow in colour. Visual selection and sub-cultures of these pheno-variants produced cultures where plantlet regenerated repeatedly and competently (Fig.1 A-D).

In teak, although, plants from tissue cultures have been regenerated on an array of basal medium such as MS (Kushaokar and Sharon, 1996; Ranasinghe and Beriyn, 1996; Muminova *et al*., 1999; Tiwari *et al*., 2002; Ramesh *et al*., 2003), B_5 medium (Ramesh *et al*., 2003) and plant woody medium (Kushaokar and Sharon, 1996; Muminova *et al*., 1999; Ramesh *et al*., 2003), in our experiments, MS basal medium was used throughout the experiments, as it was found more responsive in preliminary experimentations than others.

During course of present investigations two auxins (NAA and 2,4-D) and two cytokinins (BAP and Kinetin) were used singly (Table 1,2) as well as in a number of combinations and concentrations (Table 3,4) for culture establishment. Results clearly indicated varying response of growth regulators on callus proliferation. Higher callus initiation was observed on culture media fortified with an auxin. At lower levels both the auxins 2,4-D or NAA have been found to initiate callus proliferation, however, such calli failed to produce normal plants. At higher levels of 2,4-D ($\geq$3.0 mg L^{-1}) callus turned blackish with retarded growth whereas cell mortality was observed at $\geq$4.0 mg L^{-1} of 2,4-D. At the higher concentration ($\geq$5.0 mg L^{-1}) of NAA, most of the callus were non-morphogenic and with the ageing changed colour from cream to dark brown without any further growth. Maximum callus initiation was observed on culture medium containing NAA at 2.0-3.0 mg L^{-1}. Culture media containing NAA produced fast growing yellow and green compact callus, which showed beneficial effect on the cell growth of morphogenic cultures.

Callus produced in media supplemented with kinetin resulted in the formation of hairy roots with lower frequency of shoot formation during the advanced phase of cultures. Among five levels of cytokinins tested, lowest level (0.5mg L^{-1}) stimulated growth of morphogenic tissues. However with BA in the range of 3 to 4 mg L^{-1}, higher frequencies of morphogenic calli were observed (Table 2).

Initial studies for establishing growth regulator type, combination and concentration revealed that barely an auxin (NAA or 2, 4-D) or a cytokinin (BA or kinetin) was not much effective for morphogenic calli initiation. Mostly all the growth regulators formed calli of different sizes and texture. With a cytokinin multiple shoots proliferated from meristematic zones without intervening callus phase. Rate of recurrence of callus was higher when an auxin was supplemented to the cytokinin in culture medium. However in both the cases, frequency of morphogenic calli was found to be low. Therefore, final

experimentations were determined considering combination of an auxin and a cytokinin for achieving the best *in vitro* response. For the establishment of morphogenic cultures, the media containing NAA (2.0 mg L^{-1}) in combination with higher concentration of BA (4.0 mg L^{-1}) was found to be the most effective (Table 3, 4). These results are in accordance with various other reports on teak *in vitro* culture (Kushalkar and Sharon, 1996; Ranasinghe and Berlyn, 1996; Muminova *et al*., 1999; Tiwari *et al*., 2002; Ramesh *et al*., 2003). Medium containing 2,4-D with BAP did not initiated much of the morphogenic cultures and they fail to regenerate in the regeneration medium in spite of being large and pale yellow in colour. On the other hand, the culture media fortified with BA in combination with NAA produced yellow-green compact morphogenic callus. This phenomenon is in accordance with the findings of Ranasinghe and Berlyn (1996) and Kushalkar and Sharon (1996). During present experimentations plantlet regeneration required addition of plant growth regulators. MS basal medium supplemented with cytokinin in higher concentration and auxin in lower concentration supported plantlet regeneration in higher frequencies (Table 5; Fig.1 B). Growth regulators BA and NAA in combination elevated the plant regeneration as compared to medium with a cytokinin or an auxin alone.

Among three explants, maximum plant regeneration was observed from cotyledonary nodes followed by mature cotyledonary and epicotyl explants. Different explants of a genotype do not respond identically in cultures, most likely due to varying gradients of endogenous hormones. The plants, after hardening in the greenhouse conditions were evaluated visually. Although, detailed phenotyping was not carried out, the plants obtained from different explant cultures appeared normal and true to the type. Single explants collected from same source performed differently in culture depending upon its size and location at the donor plant. This effect yet again may be attributed to the varying endogenous hormones levels. Influence of various factors on *in vitro* response indicates that some phenomenon within the explants is as critical for a given response as its genotype (Norstog, 1970). Many of the genetic differences could be circumvented by raising the donor plants under optimal conditions as well as by varying nutrients and growth regulators in the culture medium.

Present investigation clearly suggests that cotyledonary node explant with higher regeneration potential should be used for crop improvement programme by unconventional means. Furthermore, morphogenic calli acquired from different explant cultures will be more amenable for *Agrobacterium*-mediated or mechanical gene transfer.

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Table 5 Effects of growth regulators at variable concentration on the regeneration of plantlets from diverse explant cultures.

Culture medium▼ Explants►	Growth regulators mg L^{-1}			Plantlet regeneration (%)			
	BAP	**Kn**	**NAA**	**Mature cotyledons**	**Cotyledonary nodes**	**Epicotyls**	**Mean**
MS.5B.5N	0.5	-	0.5	18.45	20.43	16.81	**18.56^{b}**
MS.5K.5N	-	0.5	0.5	14.12	17.12	11.61	**14.28^{a}**
MS.5K.5B.5N	0.5	0.5	0.5	24.19	27.26	22.28	**24.57^{cd}**
MSBKN	1.0	1.0	1.0	32.16	37.19	29.15	**32.83^{e}**
MS3B.5N	3.0	-	0.5	42.25	45.16	39.38	**42.26^{g}**
MS3BN	3.0	-	1.0	44.16	48.37	42.29	**44.89^{h}**
MS3B2N	3.0	-	2.0	47.15	51.75	44.28	**47.72^{i}**
MS4B.5N	4.0	-	0.5	55.18	57.18	52.91	**55.09^{j}**
MS4BN	4.0	-	1.0	57.16	61.38	54.19	**57.57^{j}**
MS4B2N	4.0	-	2.0	62.45	68.15	58.73	**63.11^{k}**
MS3K.5N	-	3.0	0.5	21.43	24.42	18.55	**21.46^{c}**
MS3KN	-	3.0	1.0	23.10	27.46	20.48	**23.68^{c}**
MS3K2N	-	3.0	2.0	28.49	30.95	25.65	**28.36^{d}**
MS4K.5N	-	4.0	0.5	32.16	36.57	34.73	**34.48^{e}**
MS4KN	-	4.0	1.0	37.38	39.37	34.35	**37.03^{f}**
MS4K2N	-	4.0	2.0	41.36	46.42	37.68	**41.82^{g}**
MS2N.5B	0.5	-	2.0	31.20	37.46	29.46	**32.70^{f}**
MS2NB	1.0	-	2.0	36.67	41.57	33.52	**37.25^{f}**
MS2N2B	2.0	-	2.0	41.19	46.76	39.85	**42.60^{g}**
MS2N.5K	-	0.5	2.0	20.65	26.46	18.53	**21.88^{c}**
MS2NK	-	1.0	2.0	27.68	33.18	24.76	**28.54^{d}**
MS2N2K	-	2.0	2.0	32.46	37.91	28.16	**32.84^{e}**
	Mean			**35.02^{b}**	**39.20^{c}**	**32.60^{a}**	

*Values within column followed by different letters are significantly different at 5% probability level by Duncan's Multiple Range Test.

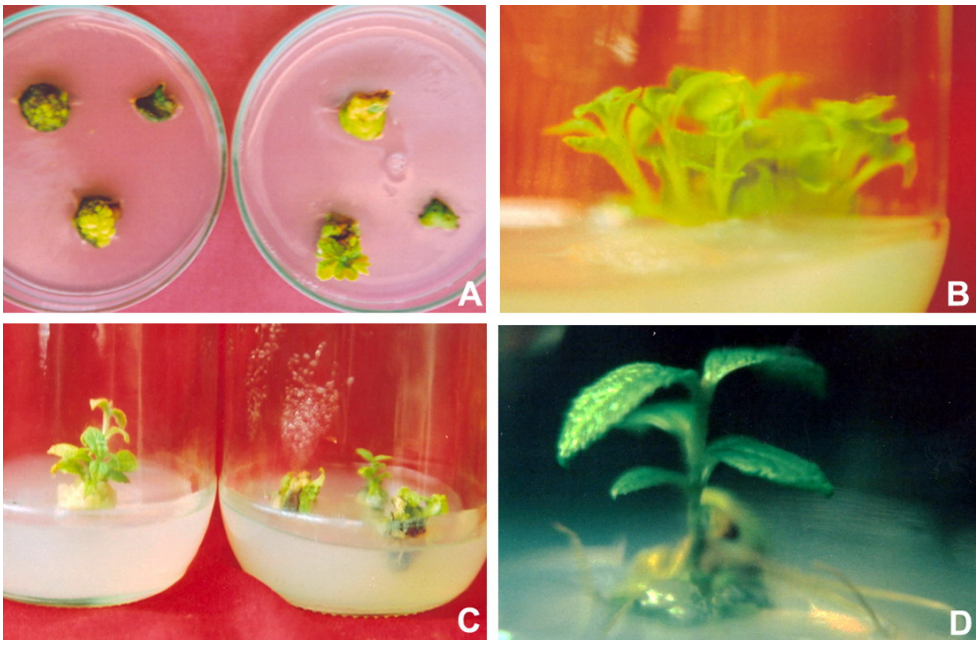

Fig.1. *In vitro* regeneration of teak (*Tectona grandis* Linn. F): A. Cultured mature cotyledons; B-C. Shoot regeneration from cultured explants; D. In *vitro* rooting in regenerants.

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Table 1. Effects of different auxins in varying concentrations on growth of various explant cultures.

Culture medium ▼ Explants▶	Conc. Mg L^{-1}	Callus induction (%)			Morphogenic calli (%)		
		Mature cotyledon	Cotyledonary node	Epicotyl	Mature cotyledon	Cotyledonary node	Epicotyl
2,4-D	0.5	52.64 ± 0.26	57.23 ± 0.28	49.32 ± 0.22	9.67± 0.08	12.32 ± 0.09	8.19 ± 0.12
	1.0	61.98 ± 0.32	64.15 ± 0.33	57.32 ± 0.25	14.23 ± 0.10	17.51± 0.11	12.63 ± 0.10
	2.0	77.23 ± 0.42	87.12 ± 0.46	84.39 ± 0.42	26.25 ± 0.16	28.13 ± 0.15	22.23 ± 0.14
	3.0	89.23 ± 0.52	81.23 ± 0.40	73.25 ± 0.36	19.56 ± 0.12	22.26 ± 0.15	17.52 ± 0.11
	4.0	84.16 ± 0.42	76.66 ± 0.46	68.25 ± 0.40	2.30 ± 0.12	1.20 ± 0.10	1.75 ± 0.12
	5.0	CM	CM	CM	CM	CM	CM
NAA	0.5	47.15 ± 0.28	49.14 ± 0.26	42.26 ± 0.22	17.53 ± 0.12	21.5 3± 0.14	15.62 ± 0.16
	1.0	52.34 ± 0.26	54.19 ±0.28	47.59 ± 0.26	24.16 ± 0.14	27.86 ± 0.15	21.26 ± 0.14
	2.0	64.18 ± 0.38	68.24 ± 0.39	56.21± 0.28	34.44 ± 0.18	37.56 ± 0.18	32.20 ± 0.18
	3.0	72.19 ± 0.36	74.15 ± 0.43	69.18 ± 0.36	32.51± 0.16	34.18 ± 0.19	30.17 ± 0.16
	4.0	80.17 ± 0.42	81.24 ± 0.45	77.32 ± 0.42	29.71± 015	35.17 ± 0.18	27.49 ± 0.18
	5.0	74.26 ± 0.38	75.36 ± 0.44	71.62 ± 0.36	2.75 ± 0.10	3.17 ± 0.12	1.81 ± 0.10

CM: Cell Mortality

Table 2. Effect of different cytokinins in varying concentrations on growth of diverse explant cultures.

Culture medium ▼ Explants►	Conc. mg L^{-1}	Callus induction (%)			Morphogenic calli (%)		
		Mature cotyledons	Cotyledonary node	Epicotyls	Mature cotyledons	Cotyledonary node	Epicotyls
BA	0.5	17.38 ± 0.12	23.16 ± 0.14	15.49 ± 0.18	14.19 ± 0.10	19.38 ± 0.16	14.16 ± 0.13
	1.0	22.54 ± 0.14	26.89 ± 0.13	18.19 ± 0.12	19.49 ± 0.13	23.59 ± 0.18	17.75 ± 0.10
	2.0	27.74 ± 0.16	29.78 ± 0.16	25.19 ± 0.15	20.16 ± 0.12	25.57 ± 0.12	18.45 ± 0.18
	3.0	32.24 ± 0.19	34.19 ± 0.18	29.16 ± 0.19	26.19 ± 0.16	28.19 ± 0.16	25.49 ± 0.13
	4.0	34.15 ± 0.22	36.16 ± 0.22	32.19 ± 0.16	32.19 ± 0.20	34.79 ± 0.22	28.49 ± 0.20
	5.0	CM	CM	CM	CM	CM	CM
Kinetin	0.5	16.32 ± 0.11	18.22 ± 0.10	14.59 ± 0.11	8.32 ± 0.10	10.88 ± 0.10	6.47 ± 0.10
	1.0	19.65 ± 0.16	20.11± 0.12	16.68 ± 0.17	11.54 ± 0.08	14.11 ± 0.12	8.38 ± 0.09
	2.0	25.96 ± 0.14	29.16 ± 0.17	23.87 ± 0.14	15.48 ± 0.11	18.52 ± 0.12	13.48 ± 0.11
	3.0	30.74 ± 0.16	34.22 ± 0.24	28.60 ± 0.24	20.45± 0.12	24.88 ± 0.15	17.76 ± 0.12
	4.0	HR	HR	HR	HR	HR	HR
	5.0	HR	HR	HR	HR	HR	HR

CM: Cell mortality; **HR**: Hairy root

Table 3 Combined effect of added auxins (at higher level) and cytokinins (at lower level) in varying concentrations and combinations on growth of teak derived from diverse explant cultures.

Culture medium▼ / Explants►	Growth regulators Conc. mg L^{-1}				Callus induction (%)			Morphogenic calli (%)		
	2,4-D	NAA	BAP	Kn	Mature cotyledons	Cotyledonary nodes	Epicotyls	Mature cotyledons	Cotyledonary nodes	Epicotyls
MSDB	1.0	-	1.0	-	68.46 ± 0.44	73.45 ± 0.43	65.86 ± 0.32	19.56 ± 0.12	24.19 ± 0.14	15.12 ± 0.11
MS2DB	2.0	-	1.0	-	75.42 ± 0.49	79.56 ± 0.47	71.12 ± 0.44	24.78 ± 0.14	28.46 ± 0.18	20.63 ± 0.14
MS3DB	3.0	-	1.0	-	80.16 ± 0.45	86.79 ± 0.52	76.36 ± 0.48	25.14 ± 0.16	32.12 ± 0.16	20.15 ± 0.12
MS3D2B	3.0	-	2.0	-	82.55 ± 0.64	84.25 ± 0.54	79.49 ± 0.56	33.46 ± 0.19	35.59 ± 0.22	29.29 ± 0.16
MSDK	1.0	-	-	1.0	64.39 ± 0.34	66.52 ± 0.34	60.31 ± 0.32	14.19 ± 0.11	20.66 ± 0.14	12.12 ± 0.10
MS2DK	2.0	-	-	1.0	69.14 ± 0.36	74.32 ± 0.42	66.32 ± 0.36	16.15 ± 0.12	18.78 ± 0.12	14.63 ± 0.10
MS3DK	3.0	-	-	1.0	80.17± 0.56	82.35 ± 0.58	77.15 ± 0.44	20.56 ± 0.13	24.46 ± 0.17	17.45 ± 0.11
MSNB	-	1.0	1.0	-	46.49 ± 0.24	47.44 ± 0.26	42.64 ± 0.28	24.36 ± 0.10	27.64 ± 0.22	18.35 ± 0.15
MS2NB	-	2.0	1.0	-	59.38 ± 0.28	63.62 ± 0.38	52.46 ± 0.36	27.23 ± 0.20	31.56 ± 0.16	24.61 ± 0.18
MS3NB	-	3.0	1.0	-	77.35 ± 0.47	79.45 ± 0.54	74.83 ± 0.40	37.15 ± 0.24	39.23 ± 0.26	32.20 ± 0.20
MS4NB	-	4.0	1.0	-	82.81 ± 0.52	87.66 ± 0.58	77.46 ± 0.43	28.23 ± 0.15	31.58 ± 0.18	24.44 ± 0.14
MS5NB	-	5.0	1.0	-	80.40 ± 0.48	86.32 ± 0.54	72.46 ± 0.40	1.88 ± 0.09	1.31 ± 0.08	1.40 ± 0.10
MSNK	-	1.0	-	1.0	42.33 ± 0.32	44.25 ± 0.28	38.14 ± 0.16	17.45 ± 0.11	20.75 ± 0.13	13.45 ± 0.11
MS2NK	-	2.0	-	1.0	55.86 ± 0.43	59.36 ± 0.31	50.19 ± 0.28	20.79 ± 0.14	24.18 ± 0.12	16.43 ± 0.15
MS3NK	-	3.0	-	1.0	72.45 ± 0.44	75.86 ± 0.46	70.34 ± 0.40	21.95 ± 0.12	25.51± 0.14	17.10 ± 0.14
MS3N2K	-	3.0	-	2.0	73.49 ± 0.46	76.23 ± 0.49	72.19 ± 0.42	28.15 ± 0.19	31.75 ± 0.18	24.14 ± 0.22
MS4NK	-	4.0	-	1.0	HR	HR	HR	HR	HR	HR
MS5NK	-	5.0	-	1.0	HR	HR	HR	HR	HR	HR

HR: Hairy root

Table 4 Combined effect of added auxins (at lower level) and cytokinins (at higher level) in varying concentrations and combinations on growth of teak derived from diverse explant cultures.

Culture medium ▼ Explants►	Growth regulators Conc. Mg L^{-1}				Callus induction (%)			Morphogenic calli (%)		
	2,4-D	NAA	BAP	Kn	Mature cotyledons	Cotyledonary nodes	Epicotyls	Mature cotyledons	Cotyledonary nodes	Epicotyls
MS2BD	1.0	-	2.0	-	67.38± 0.38	71.98 ± 0.36	65.15 ± 0.32	27.36 ± 0.14	29.56± 0.16	25.19 ± 0.13
MS3BD	1.0	-	3.0	-	64.32 ± 0.34	69.57 ± 0.32	61.15 ± .31	31.25 ± 016	33.45± 022	29.45 ± 0.16
MS4BD	1.0	-	4.0	-	60.85 ± 0.30	64.13 ± 0.31	58.24 ± 0.28	34.19 ± 0018	36.19± 0.18	32.56 ± 0.18
MS4B2D	2.0	-	4.0	-	75.45 ± 0.39	79.23 ± 0.42	71.85 ± 0.40	37.19± 0.19	41.79± 0.24	34.87± 0.20
MS5BD	1.0	-	5.0	-	CM	CM	CM	CM	CM	CM
MS2BN	-	1.0	2.0	-	63.88 ± 0.31	66.19 ± 0.46	60.19 ± 0.28	31.46 ± 0.15	33.49± 0.16	28.46 ± 0.14
MS3BN	-	1.0	3.0	-	61.25 ± 0.29	64.18 ± 0.38	56.49 ± 0.24	35.46 ± 0.18	38.87± 0.20	31.86 ± 0.16
MS3B2N	-	2.0	3.0	-	76.14 ± 0.40	79.16 ± 0.46	74.29 ± 0.38	41.58± 0.22	44.43± 0.24	38.19 ± 0.20
MS4BN	-	1.0	4.0	-	56.56 ± 0.28	60.18 ± 0.29	53.42 ± 0.25	44.86 ± 0.24	47.85± 0.28	42.65 ± 0.6
MS4B2N	-	2.0	4.0	-	71.38 ± 0.36	75.49 ± 0.38	69.15 ± 0.34	57.89 ± 0.29	61.84 ± 0.30	55.38 ± 0.24
MS5BN	-	1.0	5.0	-	68.14 ± 0.32	71.39 ± 0.30	65.45 ± 0.45	2.16 ± 0.08	1.72 ± 0.07	1.54 ± 0.10
MS2KD	1.0	-	-	2.0	62.65 ± 0.32	66.74 ± 0.37	60.43 ± 0.31	18.47 ± 0.10	20.34 ± 0.10	15.37 ± 0.10
MS3KD	1.0	-	-	3.0	57.42 ± 0.27	63.43 ± 0.32	56.19 ± 0.29	22.27 ± 0.12	24.52 ± 0.13	20.48 ± 0.12
MS4KD	1.0	-	-	4.0	54.38 ± 0.28	59.45 ± 0.27	52.38 ± 0.26	26.71 ± 0.14	30.65± 0.16	23.18 ± 0.14
MS4K2D	2.0	-	-	4.0	69.37 ± 0.36	73.48 ± 0.38	68.53 ± 0.36	37.19 ± 0.19	43.41± 0.20	32.19 ± 0.18
MS5KD	1.0	-	-	5.0	HR	HR	HR	HR	HR	HR
MS2KN	-	-	2.0	2.0	56.88 ± 0.29	60.34 ± 0.30	53.50 ± 0.30	22.34 ± 0.12	25.18± 0.13	18.18 ± 0.11
MS3KN	-	-	3.0	3.0	59.37 ± 0.27	58.45 ± 0.28	54.63 ± 0.28	24.16 ± 0.15	28.61± 0.15	20.53 ± 0.10
MS4KN	-	-	4.0	4.0	50.64 ± 0.26	56.48 ± 0.27	47.14 ± 0.22	31.65 ± 0.16	34.43± 0.18	29.18 ± 0.16
MS5KN	-	-	5.0	5.0	HR	HR	HR	HR	HR	HR

CM: Cell Mortality; **HR**: Hairy root

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