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RESEARCH ARTICLE

MORPHOLOGICAL, PHYSIOLOGICAL AND HISTOLOGICAL CHANGES IN EXPLANTS OF *TECTONA GRANDIS* LINN.F. DURING MICROPROPAGATION STAGES

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ABSTRACT

In this study, a protocol has been developed for micropropagation of *Tectona grandis* Linn.f. and studied the response of explants during tissue culture stages. The objective of this study is to understand the morphology, physiology and histology of the glandular trichomes, secretary products and Stomata. Maximum percentage of globular callus formation was observed in B_5 medium supplemented with 2,4-D 2.0 mg/l (65.4%) with activated charcoal, minimum percentage was observed in 2,4-D 0.5 mg/l (28.8%). Multiple shoots were induced from seedling node with cotyledon segments through axillary bud proliferation. Maximum percentage of multiple shoot initiation and multiplication (86.4%) was achieved on B_5 medium supplemented with BAP 2.0 mg/l and NAA 1.0 mg/l activated charcoal amended. Root induction was successfully achieved (82.4%) in B_5 medium supplemented with indole-3-butyric acid (IBA) 1.0 mg/l, the frequency of rooting of shoots were increased by previously grown on hormone-free medium. *In vitro* raised plantlets were regenerated on liquid B_5 medium supplemented with 0.5 mg/l of IAA and acclimatized on vermiculate before transferring them to field. Plantlets were regenerated on half strength medium promotes 93% of survival of *in vitro* plants. *In vitro* germinated seedlings were possesses prominent cotyledons and respond abnormally. These cotyledons were used as explants they were cultured on B_5 (Gamborg's) medium supplemented with BAP (6-benzyladenine) (3.0 mg/l), α-naphthalene acetic acid (NAA) (0.5 mg/l) with 0.3% activated charcoal. They were developed organic, globular and embryogenic calli on the surface of the cotyledons. Glandular trichomes were latter differing both anatomic as well as the composition of secretary products. The stomata present in both surface of the cotyledon and also respond permanently open.

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INTRODUCTION

Tectona grandis Linn.f. (verbinaceae) is one of the most important high quality timber yielding trees of India. Teak trees are commonly distributed in mixed forests on the alluvial beds and banks of small streams in South India and South East Asia. Teak wood is used for making fine furniture, shipbuilding, and decorative objects (Husen and Pal, 2003a). Seasoned teakwood used for many constructions and domestic purpose because of its outstanding quality of wood (Pearson and Brown, 1962). Teak is traditionally reproduced through seeds, but in most cases, germination is difficult due to the hard seed coat, low seed quality and late seed production. Poor germination rate leading to a low production of seedlings further contributes to the paucity of planting material (Tiwari *et al*., 2002; Palanisamy and Subramanian, 2001; Husen and Pal, 2003b). The propagation of teak via cuttings has been reported (Nautiyal *et al*., 1992) but this method has several limitations and only provides a few propagules from selected individuals. Various vegetative propagation methods like grafting, budding and rooting of cuttings are being practiced for teak multiplication (Goh and Monteuuis, 1997). Tissue culture in forestry has been widely studied and discussed during the past decade (Tiwari *et al*., 2002). Tissue culture technology is not only reduced the time duration and also very limited space is enough to propagate a larger number of plants throughout the year (Gupta, 1980). Micropropagation as a tool for Clonal propagation of teak to overcome these problems has been advocated (Apavatjrut *et al*., 1988). Micropropagation of teak from mature trees has remained problematic. Poor explants response and rapid explants browning are major hurdles to successful establishment of teak *in vitro*. *In vitro* propagation technique has become an efficient way for producing plants as uniform as possible on a large scale and in a short time for the plantation industry (Pandy and Brown, 2000). Micropropagation provides a high degree of phenotypic physical uniformity. Plantlets produced by tissue culture are

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usually disease free. Teak plantlets have been were achieved through multiple shoot formation from nodal segments and shoot tip explants (Devi *et al*., 1994; Shirin *et al*., 2005; De Gyves *et al*., 2007; Akram and Aftab 2008 and Senthilkumar, 2015b).

However, although numerous authors have been experimenting to establish an efficient, reproducible and simple system for micropropagation of teak (Gill *et al*., 1991; Monteuuis *et al*., 1998; Sharma et al. 2000; Khatri *et al*., 2001; Gangopadhyay *et al*., 2003; Yasodha *et al*., 2005), it still remains problematic due to the poor capacity of shoot proliferation, high susceptibility of shoots to vitrification and browning and the low frequency of *in vitro* rooting. Tissue browning *in vitro* can be a serious problem when mature shoots of trees are used as a source material for micropropagation studies (Shirin and Sarkar, 2003; Ozyigit, 2008). The present study has been clearly described that the response of *in vitro* germinated seedling node with cotyledons explants, plantlets regeneration via multiple shoots formation in B_5 medium supplemented with various hormone treatments. Morphological, physiological and histological changes during *in vitro* culture stage which has not been reported so for.

MATERIALS AND METHODS

Plant Material and Explants source

Mature teak fruits were collected from the departmental nursery, Government Arts College, Dharmapuri, Tamil Nadu, India in August 2015. Fruits were broken open the hard endocarp of mature fruit and carefully isolated the seeds. Isolated seeds were washed with running tap water for 1 hour followed by surface sterilized with 10% sodium hypochlorite for 15 minutes and 0.5% mercuric chloride for 2-3 min followed by rinsed three times in sterile distilled water in laminar air flow cabinet. The surface sterilized seeds were used for establish *in vitro* seedlings on B_5 medium.

Medium and culture condition

The nodes with cotyledons explants were excised from *in vitro* germinated seedlings. The germinated of seedlings was established on B_5 (Gamborg's) medium containing 3% sucrose and solidified with 0.8% agar. For different culture B_5 medium supplemented with different concentration of 6-benzylamino purine (BAP) (0.5-2.0 mg/l), α-naphthaleneacetic acid (NAA) (0.5-1.0 mg/l), 2,4-dichlorophenoxyacetic acid (2,4-D) (0.5-1.0 mg/l), indole acetic acid (IAA) (0.5-1.0 mg/l), and indole-3-butyric acid (IBA) (0.5-1.0 mg/l). The pH of the medium was adjusted to 5.8 with 1 N NaOH or 1 N HCl before autoclaving. The cultures were maintained at 27±2°C temperature and a photoperiod of 16/8 light/dark through standard PAR lamps. After 28 days, the aseptic seedlings were transferred to fresh medium of the same composition to study the response of explants.

Callus and Multiple shoot culture

The healthy seedlings were selected for callus and multiple shoot induction on B_5 medium supplemented with different auxin and cytokinins alone or in combinations. Carefully remove the seedlings from culture vials and trimmed into epicotyl, hypocotyl, node with cotyledon and used as explants. They were inoculated cultured on B_5 medium containing 3% sucrose and 0.8% agar supplemented with 2,4-D (0.5-2.0 mg/l) for callus induction and 0.5-2.0 mg/l of BAP was added for multiple shoot induction. The micropropagation cycle consisted of a 28 days subculture of nodal segments after removal of the new shoots onto fresh medium. Data were collected five times at a two month interval and subjected to Mean ± Standard Deviation.

Rooting, Plantlet Regeneration and Hardening

In vitro derived shoots from nodal explants were transferred to B_5 medium supplemented with different concentrations of IBA, IAA and NAA for rooting. The rooting medium consisted 3% sucrose and solidified with 0.8% of agar supplemented with Indole-3-butyric acid (IBA) was tested either alone (at 0.5, 1.0, 1.5, 2.0 mg/l) or in combination with BAP (0.5 mg/l). The aseptically grown shoots were cut into single nodes with their respective 2 leaves and placed, randomly distributed, into the different shoot proliferation media. In general, from each shoot 4-5 nodes were obtained. The cultures were initially maintained for 6 days under dark and then exposed to light. Multiple shoots were rooted by following the procedure of Akram and Aftab, (2007). All experiments were repeated for five times. The regenerated young plants were removed from the culture vials and washed thoroughly with sterile distilled water. They were acclimatized and then planted in plastic bags in culture room in a sterile soil mixture, sand, and vermiculite (1:1:1 v/v) enriched with half strength of B_5 salt solution and placed in a glass house (33±2ºC, RH 85%). Three-month-old plants were planted in the field and their survival is being observed.

Histology

The free-hand sections were taken and used to study the morphology, anatomy and histology of *in vitro* grown cotyledons. Sections were immediately stained and observed. The materials for anatomical study were fixed in Formaldehyde- Acetic acid Alcohol mixture. Histological and histochemical staining was carried out according to standard procedure (Johansen, 1940). Microphotographs were taken with a Nikon DSLR 5500 camera, microscope system having bright-field, dark-field, phase-contrast, Nomarski-DIC, polarized light and fluorescence modes. Macrophotographs were taken using a Nikon DSLR 5500 camera, Olympus stereo zoom microscope.

Data analysis

A completely randomized design was used for all experiments. Callus formation, multiple shoot initiation, root induction and plantlet regeneration were carefully calculated based on the number of explants used. Data of all experiments were statistically analyzed and expressed as Mean ± Standard Deviation (Snedecor and Cochran, 1968).

RESULTS

Seed germination

In vitro germination studies were carried out by using seeds isolated manually cut open the hard endocarp of mature dry fruits. After surface sterilization of seeds was carefully inoculated on B_5 medium supplemented with different hormone treatments as well as control was maintained in dark

and light conditions. The germination percentage was calculated based on number of seeds were used in the culture vials. Maximum seed germination (74.3%) was observed in medium supplemented with BAP 0.5 mg/l followed by IAA 0.5 and 1.0 mg/l (48.4%, 41.5%), NAA 0.5 and 1.0 mg/l (35.7%, 27.2%) respectively. The minimum percentage (14.2%) of seed germination was observed in control medium (Data were not shown). When compare the seed germination in light and dark conditions, more seed germination was achieved in light condition than in dark condition. But the biomass of seedlings was showed higher in dark than light. Seedlings from light grown were only used for the study of *in vitro* responses.

Callus culture

In vitro response of teak seedling node with cotyledon explants were cultured on B_5 medium with 3% sucrose, 0.8% agar. For mass propagation, B_5 medium supplemented with (BAP, 2,4-D, IAA, NAA) alone at the concentration of 0.5-2.0 mg/l as well as in combinations. Node explants were respond different frequencies in all hormone treatments. For callus induction, 2,4-D, IAA and NAA were respond well than BAP. Callus initiation and proliferation was noticed in all explants on B_5 medium. When compare to other hormones treatments 2,4-D (0.5 to 2.0 mg/l) showed high percentage of callus formation followed by NAA (0.5-1.0 mg/l), IAA (0.5-1.0 mg/l), respectively. Maximum percentage of callus formation was observed in B_5 medium supplemented with activated charcoal supplemented with 2,4-D 2.0 mg/l (65.4%), followed by 52.3% in 1.5 mg/l, 34.6% in 1.0 mg/l and 28.8% in 0.5 mg/l (Table 1).

Three to five days after inoculation on B_5 medium, the nodes were started to respond first to formation of callus then shoot induction. Percentage of callus initiation was calculated based on number of explants inoculated. Increasing the concentration of hormones, the response of cultures also increases up to 2.0 mg/l, above (2.0 mg/l) of concentration the explants and cultures respond abnormally. Very less amount of callus formation (5.5%) was observed in BAP 0.5 mg/l with activated charcoal amended. Well-developed callus cultures were sub-cultured on medium supplemented with same concentration of hormone. After three to five subculture, the calli were developed into as organogenic calli. From these organogenic calli were further developed into multiple shoots in subsequent subcultures.

Multiple shoot formation

Initially multiple shoots were developed from the organogenic calli as well as shoot tip cultures subculture. Two shoots per node, four to five shoots shoots were produced in shoot tip explants. The multiple shoot induction and multiplication was observed in medium supplemented with BAP (1.0-2.0 mg/l) in combination of 2,4-D, IAA or NAA (0.5-1.0 mg/l). Maximum numbers of shoots (86.4%) were induced on B_5 medium supplemented with BAP 2.0 mg/l and NAA 1.0 mg/l. Similarly the initiation of multiple shoots was also achieved in other combination of BAP (1.0-2.0 mg/l) in combination of IAA (0.5-1.0 mg/l). The frequency of shoot formation was less (12.3%) in B_5 medium supplemented with BAP 0.5 mg/l and 2,4-D 0.5 mg/l hormone treatment.

Table 1. Response of node with cotyledon and shoot tip explants of *Tectona grandis* Linn.f. on different hormones supplemented with B_5 medium

Hormones (mg/l)	Callus Induction (%)	Multiple shoots (%)	Root induction (%)	Plantlet regeneration (%)
Control	3.2 ± 0.3	-	-	-
BAP 0.5	5.5 ± 0.2	18.2 ± 1.3	-	3.5 ± 0.2
BAP 1.0	7.5 ± 1.2	24.1 ± 1.6	-	5.1 ± 0.5
BAP 1.5	8.5 ± 0.7	28.6 ± 2.0	-	8.4 ± 0.6
BAP 2.0	10.6 ± 1.5	32.1 ± 2.8	-	12.6 ± 1.0
2,4-D 0.5	28.8 ± 2.2	-	-	-
2,4-D 1.0	34.6 ± 2.8	-	-	-
2,4-D 1.5	52.3 ± 3.4	-	-	-
2,4-D 2.0	65.4 ± 4.2	-	-	-
IAA 0.5	8.7 ± 0.4	-	28.4 ± 2.2	10.5 ± 0.3
IAA 1.0	15.3 ± 0.7	-	32.4 ± 1.8	15.6 ± 1.4
NAA 0.5	7.5 ± 0.4	-	40.1 ± 2.0	-
NAA 1.0	13.2 ± 1.2	-	48.3 ± 2.1	-
IBA 0.5	6.5 ± 0.4	-	70.5 ± 5.1	-
IBA 1.0	11.5 ± 1.2	-	82.4 ± 6.0	-
BAP 0.5 + 2,4-D 0.5	20.1 ± 2.3	12.3 ± 1.1	-	-
BAP 0.5 + 2,4-D 1.0	24.3 ± 2.0	16.4 ± 1.2	-	-
BAP 0.5 + 2,4-D 1.5	30.1 ± 2.4	18.5 ± 1.8	-	-
BAP 0.5 + 2,4-D 2.0	34.6 ± 2.5	22.0 ± 2.2	-	-
BAP 0.5 + IAA 0.5	10.2 ± 0.5	28.3 ± 2.1	3.8 ± 0.2	18.7 ± 1.6
BAP 0.5 + IAA 1.0	13.4 ± 0.9	32.1 ± 3.0	4.3 ± 0.3	25.3 ± 2.1
BAP 1.0 + IAA 0.5	16.2 ± 1.0	36.2 ± 3.2	5.3 ± 0.4	28.6 ± 2.5
BAP 1.0 + IAA 1.0	21.0 ± 1.2	46.2 ± 2.6	6.2 ± 0.5	35.2 ± 3.4
BAP 0.5 + NAA 0.5	3.3 ± 0.1	43.3 ± 4.1	3.2 ± 0.2	36.5 ± 3.2
BAP 0.5 + NAA 1.0	4.5 ± 0.3	55.1 ± 4.8	4.1 ± 0.3	48.2 ± 4.5
BAP 1.0 + NAA 0.5	6.6 ± 0.5	52.1 ± 4.7	6.3 ± 0.5	56.2 ± 5.2
BAP 1.0 + NAA 1.0	8.5 ± 0.6	66.7 ± 5.2	8.8 ± 0.6	61.5 ± 5.6
BAP 1.5 + NAA 0.5	10.4 ± 1.2	62.1 ± 5.5	6.3 ± 0.4	67.2 ± 5.5
BAP 1.5 + NAA 1.0	12.8 ± 1.3	80.3 ± 6.4	8.5 ± 0.6	72.4 ± 6.6
BAP 2.0 + NAA 0.5	13.7 ± 1.2	77.2 ± 6.6	12.0 ± 1.0	82.3 ± 7.1
BAP 2.0 + NAA 1.0	17.4 ± 1.4	86.4 ± 6.5	15.2 ± 1.2	86.2 ± 7.7

Mean ± S.E for five experiments

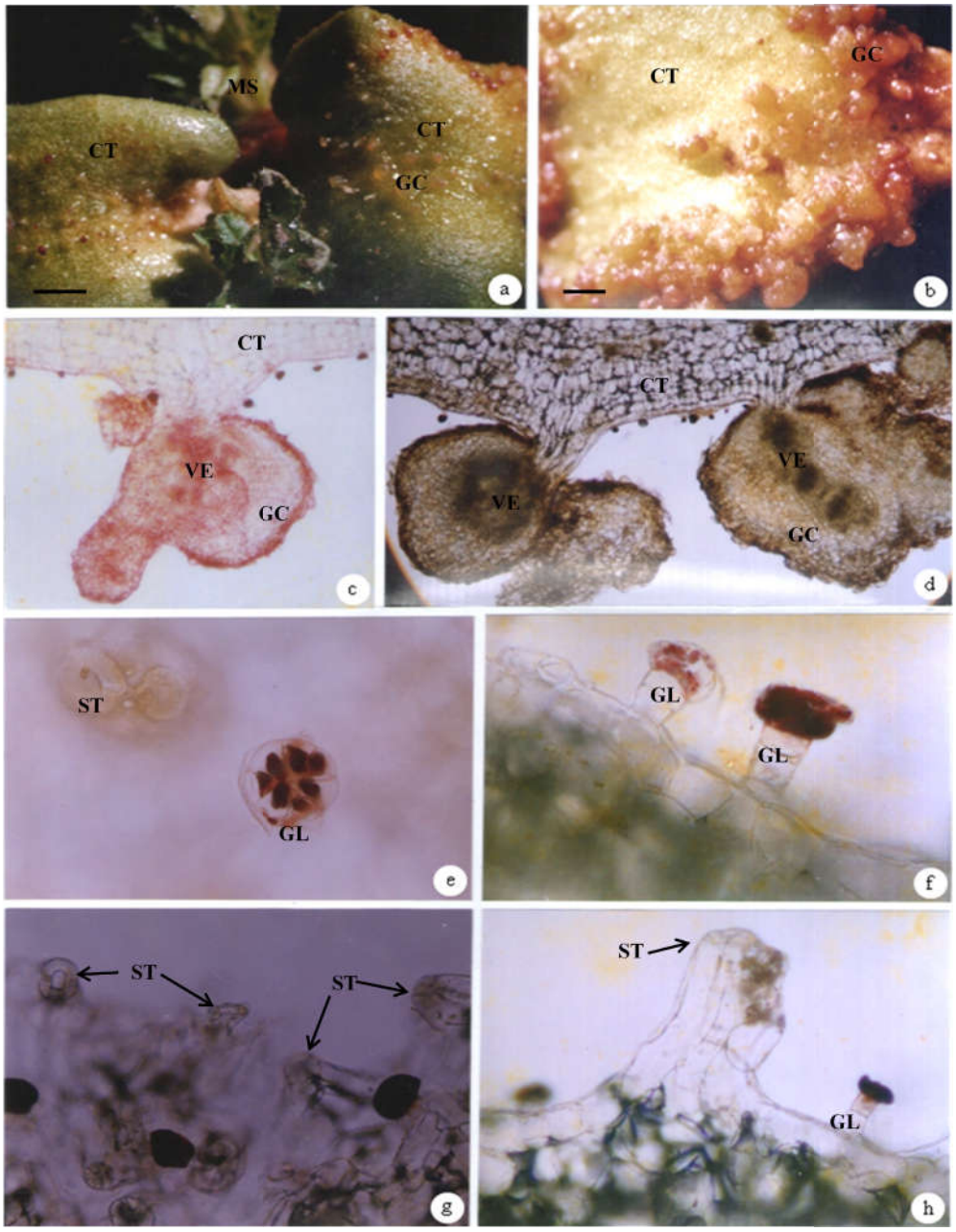

Figure 1. ***In vitro*** **germinated teak seedling cultures showed the morphological, physiological and anatomical changes. a. Multiple shoots initiation from the nodes. b. The globular callus development on the surface of cotyledon. c. Transverse section of tissue cultured cotyledon showing globular callus proliferation and multicellular glands (300X). d. Transverse section showing globular callus with vascular element at the centre (300X). e & f. The multi-cellular glands showing dye accumulating heads developed in the surface of the cotyledons. g & h. Raised and permanently opened stomata shown in** ***in vitro*** **grown cotyledons of teak. (bars in a & b: 1 mm) (CT-cotyledon, MS-multiple shoot, GC-globular callus, VE-vascular elements, GL-multicellular gland, ST-Stomata)**

In vitro Rooting

After four to five weeks of shoot cultures were transferred to rooting medium with the presence of different auxins. For rooting of shoots B_5 medium supplemented with three auxins IBA, IAA and NAA (0.5-1.0 mg/l) alone in different concentrations. When compared the different auxin treatments B_5 medium amended with IBA 1.0 mg/l showed (82.4%) followed by NAA 1.0 mg/l (48.4%) and IAA 1.0 mg/l (32.4%). Rooting was significantly affected with the increasing concentration either (1.0 mg/l) of IBA, IAA or NAA. IBA alone induced 60% rooting depending on its concentration, producing swollen and brittle roots. Callus formation at the base of shoots increased with increasing concentrations of IBA above the level of (1.5-2.0 mg/l). The number of roots per explant was low or absent with the lowest IBA (0.5 mg/l) concentration. The use of a low concentration of IBA (1.0 mg/l) is advisable since it does not interfere with shoot growth, root elongation and keeps the basal callus. However, it induces only a few thin roots without lateral ramifications. Roots emerged at the base of shoots after 2 weeks. An additional 2 weeks on the same hormone supplemented B_5 medium increased the number of roots as well as promoted shoot elongation.

Micropropagation and acclimatization

After root initiation, the regenerated plantlets were removed from the culture vials and washed with sterile distilled water to remove agar sticking in the roots. Plantlets with fully expanded leaves and well-grown roots (21 d) were transferred to polythene bags containing sterile soil, sand, and farmyard

manure (1:1:1 v/v). *In vitro* raised plantlets were regenerated on liquid B_5 medium supplemented with 0.5 mg l^{-1} of IAA and acclimatized on vermiculate before transferring them to field. This method has been resulted in 97.9% survival of the plantlets. For acclimatization of micropropagated plants were kept under 16/8 photoperiod at 26±2°C and regularly poured half strength B_5 solution for one month. Relative humidity (80%) was maintained by covering them with polythene bags. All the transferred plant were then hardened and later established in the field successfully.

Response of cultures

In the present study, the morphologic, physiologic and histological responses of explants were studied under identical cultural conditions. *In vitro* teak seedlings were used as explants for mass multiplication. The explants such as node containing cotyledons were responded well in all treatments. Initially the cotyledons become green and larger size (Fig. a). The surface of cotyledons were initiated numerous outgrowths, they were developed into small globular callus later culture stages (Fig. b). The morphological changes of cultures were responded physiologically different. The anatomical observations were clearly indicate that the origin of globular calli from the surface of cotyledon. The globular calli were developed either from trichomes, multicellular glands or stomata. Free hand sections of cotyledon were showed globular callus proliferation from the epidermal regions of cotyledon. Globular callus showed the mass of cells and vascular elements at the centre (Fig. c, d). They were further developed into individual shoots. The presence of multicellular glands in *in-vitro* cultures has not been previously properly examined. Each gland has a stalk and a head consisting of eight cells. The head cells secrete and accumulate a red pigment which appears finally granular at high magnifications. Occasionally within each head the pigment occurs as small droplets. These dye-secreting glands also proliferate and lead to development of callus (Fig e). The presence of these glands in tissue culture, and the elongation of their stalk cells suggest that it may be possible to manipulate these glands to produce large quantities of this dye under *in-vitro* conditions. Glandular trichomes consisted of an epidermic basal cell, a uniseriate stalk and a unicellular secretory head. The stalk can be short, formed by a single cell, or long with up to four cells; and the upper cell showed a special type of differentiation, as a neck cell (Fig. f). Numerous stomata also present on the surface of cotyledon, they were permanently open (Fig. g) and raised from the surface (Fig. h). Each stomatal apparatus has kidney shaped guard cells characteristic of dicot stomata.

DISCUSSION

Teak seeds were germinated between 8 to 12 days after inoculation on medium. The previous *in vitro* germination studies have indicated that the viability is very low in teak (Gyves *et al*., 2001). Teak explants of nodes and shoot tips were cultured on B_5 medium with 3% sucrose, 0.8% agar and 0.3% activated charcoal and without charcoal for callus initiation, multiple shoot formation and plantlet regeneration with suitable hormones. Callus initiation and proliferation was achieved in medium supplemented with 2,4-D. Similarly stem callus proliferation was obtained on MS medium supplemented with 2.0 mg/l of 2,4-D in *Withania somnifera* (Manickan *et al*., 2001). Maximum callus growth was noticed within 6 weeks of culture in MS medium supplemented with BAP 2.0 mg/l and KIN 1.0 mg/l in *Melia azadarech* (Thakur *et al*., 1998). Plant hormone 2,4-D has been found very effective callus formation and organogenesis in *Saccharum officinarum* variety Co 740 (Mohatkar *et al*., 1993). The regeneration obtained with only BA containing media were stunted, hard, short internodes with comparatively small leaves. BAP and KIN supplemented medium produced normal dark green and broad leaves with long internodal space and showed better growth. Similar results were observed in *Tylophora indica* (Faisal and Anis, 2003) and *Wattakaka volublis* (Chakradhar, 2004). The synergistic effect of cytokinin and auxin has been reported in teak (Shirin *et al*., 2005). Shirin *et al*., (2005) also achieved multiple shoots on BA (10 mg/l) and NAA (1.0 mg/l) using nodal explants from mature teak shoots. The process of teak culture establishment in this experiment correlated with Devi *et al*., (1994); Gyves *et al*., (2007) and Gupta *et al*., (1980), who reported that axillary shoots developed after 7-10 days and with Tewari (1992) who observed axillary shoots after 6 weeks on several nodes. The multiple shoots initiation and multiplication was observed in medium supplemented with BAP (1.0-2.0 mg/l) in combination of NAA and IAA (0.5-1.0 mg/l). From the results of the present investigation, it is evident that shoots from large stem segments is a strong possibility for recalcitrant woody plant species of tropical origin. According to the literature, BAP has been the most commonly used in micropropagation of teak alone or with kinetin (Gill *et al*., 1991; Goswami *et al*., 1999, Srinivasan *et al*., 2012). However, high concentrations of BAP can be inhibitory to the growth of axillary bud sprouts and can present some risks of unexpected abnormalities of new shoots after this treatment such as vitrification. The synergistic effect of cytokinin and auxin has been re-ported in teak (De Gyves *et al*., 2007). The shoot multiplication is enhanced by subsequent cultures the observations in this study are in agreement with reports such as *Gymnema sylvestre* (Komalavalli and Rao, 2000), *Hemidesmus indicus* (Sreekumar *et al*., 2000) and *Holostemma ada kodien* (Martin, 2002).

Shirin *et al*. (2005) also achieved highest number of multiple shoots on BAP and NAA using nodal explants from mature teak shoots. Gangopadhyay *et al*., (2003); Nissen and Sutter (1990) carried out rooting in a liquid medium supplemented with IBA and IAA, but the percentage of the survival of plants after transplantation was relatively low. The best rooting was observed in IBA in B_5 medium than NAA. Rooting was significantly affected with the increasing concentration of either IBA or NAA. Our results offer better performance in terms of rooting percentage and plant survival and rapid growth. Singh *et al*., (2006) used coppice shoots that were rooted with IBA and thiamine with 38.3% rooting response. In all the above reports, only IBA was used for rooting of coppice as well as mature shoot cuttings of teak. Teak seedlings have been responding abnormally in different culture stages of micropropagation. From the point of view, tissue cultured explants have various trichomes and stomata are have a significant role in morphogenesis. During morphogenesis of tissue cultured organs have to develop their external parts properly. Otherwise, the tissue cultured organs may not survive in next step of culture stages. The different types of trichomes were found such as conical, unicellular, with thick and warty wall and calcified apical portion. In young leaves, the non-glandular trichomes partially obscured the glandular trichomes and it appeared that they matured at an early stage of leaf development (Senthilkumar, 2015a). When fully mature gland comparatively large in size and filled with dye. Occasionally

within each head the pigment occurs as small droplets. However, the red dye has been extracted and commonly utilized for natural dying of fabric cloth (Chandra, 1976). Two dye-accumulating glands develop from the cotyledon in tissue culture-derived tissue.

Two major physiological causes for rapid water loss resulting in low survival rates of tissue cultured plants have been attributed: high rate of cuticular transpiration due to poor development of epicuticular wax and lack of stomatal control (Santamaria *et al*., 1993). Thus, it is possible that the proliferated multicellular glands and subsidiary cells of the stomata can lead to the establishment of callus on the surface of cotyledonary leaves. It is interesting to note that in *in-vitro* culture of neem. Ravichandran (1996) had demonstrated that multicellular resin glands and the earliest structures to develop and elongate from the surface of the callus tissue and multiple shoots. Our results showed that more than 800 shoots can be obtained in a similar period of time, through the dissection of the shoots into nodal explants avoiding a further subculture onto shoot elongation medium to prepare shoots for rooting phase. A protocol for micropropagation of teak using B_5 basal medium supplemented with BAP and kinetin was suggested by Goswami *et al*. (1999). Acclimatization Plantlets with well developed roots were successfully acclimatized and eventually established in a green house. Acclimatization of these *in vitro* plants was done in mist chamber where high humidity is maintained also gave the better survival percentage (95%). Gradual exposure of plants from polythene covering, which was used to conserve and develop proper balance of relative humidity during the establishment of these plants helped to ensure weaning as for as humidity concerned. Gradual removal of the polythene covering day by day during the acclimatization was also reported in Verbena and Tenera (Hosaki and Katanira, 1994) for better survival. This study provides an efficient *in vitro* propagation method as well as the response of cultures during micropropagation for teak, by providing a protocol for producing genetically uniform plants from selected genotypes. Investigations were showed the highest number of micropropagated shoots reported for teak, up to now, in the literature and in a relative short period of time, producing about 4 shoots with 4 nodes within 4 weeks. The response of cultures indicates the development of external parts of the *in vitro* condition is highly useful for accalimatization phase for better survival.

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