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ADVANCES IN MICROPROPAGATION OF TEAK (*Tectona grandis* L.f).

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ABSTRACT

A successful procedure has been developed for the In vitro propagation of Teak. The explants were collected in the month of March. Sterilization of the explants was done with the use of both 10 % Sodium Hypochlorite & 1% solution of Mercuric Chloride. After the proper sterilization, the explants were trimmed & inoculated in the establishment medium (MS Basal medium + Adso4 10mg/l + Citric Acid 40 mg/l + activated charcoal 0.1% + Sugar 30 g + ph -5.7). After successful cultures establishment, the cultures were transferred to the various trails of shoot multiplication medium. Among the various trials the trail code T3 (½ MS medium + BAP 1.5 mg/l + IAA 0.05 mg/l + Kinetin 0.5 mg/l + Sucrose 30 g/l+ Agar 5.7 g + ph 5.8) showed the excellent result with 4.6 mean no of shoots with mean height of 5.8 cm in four week time . The shoots were healthy, leaves were properly opened, and no sign of vitrification was noticed. For root induction various trails were conducted, among the trail code RT4 (½ MS basal medium +1mg/l NAA + Sucrose 30g/l + Agar 5.8g/l + ph 5.8) showed the best result, with 98% rooting in four week time. Well elongated & rooted cultures were selected for the acclimatization. For proper hardening two steps method of hardening was conducted. In primary hardening the plants were transplanted in pro-tray filled with coco-peat & sand. Plants were kept in 90 % humidity. After 1.5 month the when the plants were primarily hardened, then again plants were transferred to the secondary hardening medium (Coco peat+ Red soil+ coco peat) in the ratio of 1:1:1.). After 1.5 months the plants were properly hardened & developed for the field plantation. This method was found very satisfactory for the quality mass production of teak planting materials in short period of time with excellent morphology.

KEYWORDS : BAP- 6-Benzyle Amino Purine, IAA- Indol Acetic Acid, Micropropagation, NAA- Naphthalene Acetic Acid

Teak (*Tectona grandis* L.f) is one of the best Known most valuable timbers of tropical region. It is also valued for its unique timber quality all around the world. The main feature of teak plant is because of durability & it also has very unique mark of wood after maturity. It belongs to Verbanaceae family.

The attraction of the species is the mechanical & physical quality of its timber which is also resistance of termites attack due to the presence of sesquiterpenes (Anon, 1996; Maslekar,1983).

It is native to India, Myanmar, Laos and Thailand, is one of the world's premier hardwood timber, attraction for its mellow color, fine grains & durability (Kommalapati, 2009). At present teak ranks among the top five tropical hardwood species in terms of plantation area established worldwide (Dah and Baw, 2001, Monteuuis et al., 1998).

Poor germination rate, low fertility of soil, leading to low production of seedling further Contributes to the paucity of planting material (Tiwari et al., 2002). Planting stock production is by seed are main disadvantage, mainly poor fruit production, empty fruit & a low germination rating (Gupat and Kumar, 1976).

Commercial In *vitro* techniques are the most efficient method to produce the quality true to type planting material in short time. As advocated earlier, In vitro propagation technique has become as efficient way for production plant as uniform as possible on large scale in short time for the plantation industry. It was also stated that the Micropropagation offers a rapid means of aforestation, multiplying woody biomass, conservation of elite and rare germplasm (Bajaj, 1986; Karp, 1994). In the present the study, the research has been conducted to find the efficient way of in *vitro* fast multiplication of true to type planting material in short period of time.

MATERIALS AND METEHODS

Collection of Explants and Sterilization

New emerging buds were collected in the month of March. The apical buds and nodal segments were taken from both juvenile & mature tree. Care was taken while collection of explants to avoid contamination. After the primary tap-water wash, for 10 minutes, the explants were kept in 10% solution of Ascorbic acid for two hours of duration. Then explants were trimmed to the appropriate size of 1 cm to 5 cm. Then after explants were kept in the solution of systematic antifungal agent, (Bivastin 10 mg/l), for half an hour, followed by tap water wash for 15 to 20 minutes. Then explants were taken inside to the lab in semi sterile area where, the explants were treated with 10

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% solution of sodium hypochlorite for 15 minutes with few drops of tween -20, followed by 5 times wash in Double distilled water. Then after explants were taken inside the laminar flow bench for further sterilization. Finally buds were treated with 1% solution of Mercuric Chloride for 10 minutes followed by 5 times wash in double distilled water.

Culture Establishment

Single buds were placed in MS basal medium (Murashige & Skoog, 1962) with Adenine Sulphate, 10 mg /l, + Citric acid, 40 mg/l, + Activated charcoal 0.1 %, + Sugar 30 gm/l + Agar 6 g/l. The medium was prepared with the temperature of 121 degree centigrade with 15 psi, for 18 minutes of duration. The ph of the medium was set to 5.8 with 1N HCL & 1N NAOH. 40 ml medium was taken per container .The Apical Buds & nodal segments both were taken for the inoculation. The cultures were incubated for the time period of 5 to 6 weeks, in the controlled temperature of 25 ± 2 degree centigrade. The 16 hour photoperiod were provided with 60 % relative humidity. Cultures were frequently transferred to the fresh medium to avoid blackenening because of excessive secretion of tannin.

Multiplication of Cultures

After the successful establishment of cultures after the four weeks. The contamination free cultures were transferred to the various medium of different combination trails for the shoot multiplication. The nodal & apical segments both were taken, leaves were trimmed & inoculated in medium.

Five medium trails were conducted each with full & Half strength Basal MS medium with BAP (6- Benzyl aminopurin) at the concentration ranging from 0.5 mg/l to 2.5 mg/l , IAA concentration ranging from 0.05 mg/l , Kinetin 0.25 mg/l , 0.5 mg/l, Agar , 5.8 mg/l , & Sucrose 30 g/l with ph 5.8.

For each experiment five replications were done with two repetitions, for the authenticity of the observation. The cultures were incubated for 5 weeks of time period, in the controlled temperature of 25±2 degree centigrade. The 16 hour photoperiod was provided with light intensity of 3000 lux. The relative humidity was maintained to 60%.

Rooting

For rooting eight medium trails were conducted with both half & full strength of MS basal Medium. In all the trails two potent Auxin (IAA & NAA) were used alternatively in the concentration of 0.5 mg/l & 1mg/l. with Sucrose 30g/l + Agar 5.7 g/l + ph 5.8. Cultures were incubated in the light intensity of 3000 3500 lux, for the 16 hour photoperiod, with temperature of 25 ± 2. The relative humidity was maintained to 55 to 60 % & 4 weeks of time period were provided for rooting.

Acclimatization

Well elongated & rooted plants were selected. Two stage hardening was conducted.

a. Primary hardening

b. Secondary hardening.

Primary Hardening

The well elongated & rooted plants were selected & graded according to quality & size. The selected Ex-Agar plants were dipped in 1% solution of Diathane M45 for half an hour, long roots were trimmed slightly to the size of approx 2- 3 cm , then the plants were transplanted in the portray filled with coco peat and sand in the ratio of 1:1. Finally plants were kept under poly-tunnel with 90% humidity for 1.5 month duration. Tunnel temperature was strictly maintained to 30 to 35 degree centigrade with white cloth on poly tunnel to avoid temperature shock. The intermittent misting was done. For the precautionary measure, the antifungal solution was sprayed on weekly basis. After two weeks the tunnel humidity was gradually reduced to 50 %, by the half opening of the tunnel.

Secondary Hardening

After the primary Hardening, the plants was transplanted to the mixture of secondary hardening substrates (Coco-peat+ red soil+ vermicompost) in the ratio of (1:1:1). The mixture was filled in black-poly bags, after transplantation, the plants were kept under 50% agro shade net of green color. Slight fertilizers (NPK) were sprayed on interment basis, after one week of transplantation. Plants were kept in the weeds free green house.

Figure 1 : Initiation

Figure 2 : Culture Establishment

Figure 3 : Trail Code T1

Figure 4 : Trail Code T2

Figure 5 : Trail Code T3

Figure 6 : Trail Code T4

Figure 7 : Trail Code T5

Figure 8 : Trail Code T6

Figure 9 : Trail Code T7

Figure 10 : Trail Code T8

Figure 11 : Trail Code T9

Figure 12 : Trail Code T10

Figure 1 : Initiation
Figure 2 : Successful Establishment of Culture
Figure 3 : (T1- (1/2 MS+0.5 BA+0.05 IAA+0.5 KIN)
Figure 4 : (T2- (1/2 MS+ 1 BA+0.1IAA+0.5 KIN)
Figure 5 : (T3 (1/2 MS+1.5BA+0.15 IAA+0.5 KIN)
Figure 6 : (T4- (1/2 MS+2 BA+ 0.2 IAA+0.5 KIN)
Figure 7 : (T5- (1/2 MS+2.5 BA+0.25 IAA+0.5 KIN)
Figure 8 : (T6- (MS+0.5 BA+0.05 IAA+0.5 KIN)
Figure 9 : (T7- (MS+1 BA+0.1 IAA+0.5 KIN)
Figure 10 : (T8- (MS+1.5 BA+0.15 IAA+0.5 KIN)
Figure 11 : (T9- (MS+2 BA+0.2 IAA+0.5 KIN)
Figure 12 : (T10- (MS+2.5 BA+0.25 IAA+0.5 KIN)

Figure 13 : Teak Rooting

Figure 14 : Root Development

Figure 15 : Rooted Plant in Media

Figure 16 : Ex -Agar Rooted Plants Ready for Hardening

Figure 17 : Hardened Plants Ready for Field Plantation

Note : Rooting Figure Shown Above, Only The Best Combination (½ MS + 1mg/l NAA)

Table 1 : Trail A. Multiplication Stage

Trail code	MS (Strength)	BAP (mg/l)	IAA (mg/l)	Kinetin (mg/l)	Mean no of shoots ± SE	Mean height of shoots in cm ± SE
T1	½	0.5	0.05	0.5	1.4 ± 0.21	1.4 ± 0.21
T2	½	1	0.1	0.5	2.2 ± 0.33	1.8 ± 0.33
T3	½	1.5	0.15	0.5	4.6 ± 0.21	5.8 ± 0.17
T4	½	2	0.2	0.5	4.4±0.52	1.2 ± 0.17
T5	½	2.5	0.25	0.5	11.6±0.83	0.8 ± 0.10

*SE Standard Error

Table 2 : Trail B. Multiplication Stage

Trail code	MS (Strength)	BAP (mg/l)	IAA (mg/l)	Kinetin (mg/l)	Mean no of shoots ± SE	Mean height of shoots in Cm ± SE
T1	Full	0.5	0.05	0.5	1.4 ± 0.21	0.7 ± 0.13
T2	Full	1	0.1	0.5	1.4 ± 0.21	1.0 ± 0.08
T3	Full	1.5	0.15	0.5	4.8 ± 0.33	3.6 ± 0.21
T4	Full	2	0.2	0.5	8.2 ± 0.17	0.5 ± 0.9
T5	Full	2.5	0.25	0.5	13.4 ± 0.9	0.16 ± 0.03

*SE Standard Error

Table 3 : Rooting

Trail Code	MS medium (strength)	IAA conc. (mg/l)	NAA (mg/l)	% of Healthy roots	Leaves health
RT1	½	0.5	-------	10	H , O
RT2	½	--------	0.5	30	H
RT3	½	1	--------	30	H
RT4	½	--------	1	98	H, O
RT5	full	0.5	-------	40	H
RT6	full	-------	0.5	40	H
RT7	full	1	------	25	V
RT8	full	-------	1	75	V,O

*H- Healthy, *V- Vitrification, *O- Opened

RESULTS AND DISCUSSION

The Explants were taken from both mature tree & juvenile green house plants. It was noticed that both have showed the same result. It was also advocated earlier that the plantlets can be regenerated from both juvenile & mature trees (Dunstan, 1988; Thorpe et al., 1991). But the cultures darkening were more prominent in mature explants. To avoid darkening of cultures because of phenol exudation the explants were kept in antioxidant .It was also advocated earlier that the phenolic compounds exudates cause necrosis and death in some explants in In vitro condition. It is hence a pre requisite to remove these compounds from explants before culturing to avoid medium darkening. (Pandey et al, 2006.) After the proper sterilization the cultures were inoculated in initiation medium Result represented in table 1, 2 and 3.

Multiplication

For multiplication of shoots ,total 10 trails were conducted, five trials each of half & full strength MS medium with 6- Benzyl amino purine (Conc. ranging from (0.5, 1, 1.5, 2, 2.5) mg/l + Idol Acetic Acid (conc. ranging from (0.05, 1, 0.15, 0.2.0.25) mg/l + Kinetin 0.5 mg/l + Agar 5.8 + Sucrose 30 g/l. The earlier report also advocated the synergistic effect of both cytokinin & Auxin in teak. (Shirin et al., 2005). Surrender & Narender (2009) also reported the uses of MS medium with cytokinin & auxin for the multiple shoot induction. Rout et al., (2008) also reported the induction of multiple shoots on Use of BA, Ads & IAA. The optimum gelling strength was found with the concentration of 5.8 g/l agar, which favored the best growth & no sign of vitrification. It is also stated earlier that the number of shoots, growth of shoots & morphology greatly affected by the concentration of Cytokinin & gelling agent's strength. (Yosodha et al., 2005).Among all the trial the trial code (T3) of half strength MS medium showed the most satisfactory result with 4.6 mean shoots with 5.8 cm mean height. The plant shoot girth was also good with moderate callusing; no sign of vitrification & leaves were healthy & properly opened. Inter nodal gap was also satisfactory. No sign of malformed shoots were noticed. On increasing the concentration of growth regulators with half & full strength MS medium , the no of shoots increased vigorously with shunted growth, leaves were not properly opened, verification & profuse callusing was also noticed.

Rooting

For the proper root induction, well elongated shoots were taken. Various trails were done with each of half & full strength MS basal medium with both IAA and NAA with the conc. 0.5 mg/l & 1mg/l alternatively. Among the various trails code RT4 (½ MS+ 0.5 mg/l NAA+ Sucrose 30g/l + Agar 5.7 g/l + ph 5.8) was found the best one which gave 98% response with well strong & healthy roots in four weeks of time period. It was also noticed that the plants were elongating well after transferred to the rooting medium. Leaves were found properly opened & no sign of vitrification was noticed.

Acclimatization

For the acclimatization of rooted plants, two stage hardening was conducted with gradual exposure of the plants to the external environment for better hardening. It was also stated earlier that the in vitro plants lacks necessary anatomical features to withstand variation in the natural environment. Gradual acclimatization was resulted the 95% survival with best growth & morphology.

CONCLUSION

It is concluded that the above work is the authentic work which is conducted in state of art facility. The protocol is very helpful for the mass propagation of quality planting material in short period of time.

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