Indian J. Plant Physiol., Vol. 13, No. 3, (N.S.) pp. 258-265 (July-Sept., 2008)

PLANT REGENERATION OF *GMELINA ARBOREA* ROXB. FROM COTYLEDONARY NODE EXPLANTS

PRIYA RANJAN BEHERA, PRANATI NAYAK, M. THIRUNAVOUKKARASU* AND SATYABRATA SAHOO

Plant Biotechnology Laboratory, Natural Products Department, Institute of Minerals and Materials Technology (CSIR), Bhubaneswar-751 013, India

Received on 1 April, 2008, Revised on 23 July, 2008

SUMMARY

A regeneration protocol has been developed for *Gmelina arborea* Roxb. using cotyledonary nodes. Multiple shoots were induced on Murashige and Skoog's (MS) medium supplemented with 6-benzyladenine (BA) and thidiazuron (TDZ) either alone or in combination with either indole-3-acetic acid (IAA) or α–napthalene acetic acid (NAA). Cotyledonary nodes cultured on medium with BA (4.4 μM) + IAA (1.4 μM) produced higher number of shoots. Cultures maintained in TDZ supplemented medium showed poor response. *In vitro* regenerated shoots were cultured on to root induction medium consisting of half-strength MS supplemented with IAA or indole-3-butyric acid (IBA) either alone or in combination with 2, 3, 5-triiodobenzoic acid (TIBA). Rooting was best in the medium supplemented with 4.9 μM IBA + 2.0 μM TIBA. Rooted plantlets were acclimatized and transferred to the field with 70 % survival rate.

Key words: **BA, cotyledonary node, *Gmelina arborea*, IBA, micropropagation**

INTRODUCTION

Gmelina arborea Roxb. Commonly known as 'White teak' is a tree species native to tropical and subtropical regions of Asia. It has gained widespread acceptance as an exotic plantation species because of its rapid growth and wide variety of uses (Dvorak 2003). It is often planted as an ornamental avenue shade tree. The wood makes a fairly good charcoal. According to Little (1983), the leaves are harvested for fodder for animals and silkworms; occasionally man consumes the bittersweet fruits. The whole plant parts are used for the treatment of various ailments like tuberculosis, gonorrhoea, cough and headache and for ulcers (Chopra *et al*. 1956). Many chemically active compounds have been isolated from wood, leaves and roots (Rao and Rao 1970, Joshi *et al*. 1977, Satyanarayana and Rao 1986). Because of its very rapid growth, ease of establishment and early returns, the attention of foresters' world over is drawn towards this important multipurpose tree (Arya and Haque 1982).

Although traditionally *G. arborea* is propagated through seeds, seeds are not always easily available and their germination is also poor (Surendran 1990, Thirunavoukkarasu and Debata 1998). Further, their period of viability is very short. To meet the growing demand for wood and pharmaceutical based industries, it is found necessary to multiply this species by adopting *in vitro* techniques. Although propagation by means of stump sprouts (Subramanian *et al.* 1992) is possible, the conventional means of propagation do not meet the present demand. Therefore, for rapid multiplication of trees micropropagation is increasingly applied to supplement conventional methods of vegetative propagation. There are very few reports available on the

*Corresponding author, E-mail: mtarasu@yahoo.com

micropropagation of this species (Roy 1995, Kannan and Jasrai 1996, Thirunavoukkarasu and Debata 1998). The present study was undertaken with an aim of establishing an efficient protocol for *in vitro* plant regeneration from cotyledonary node explants of *in vitro* grown *G. arborea* seedlings. Experiments are afoot to find out if explants obtained from other parts of the plant serve better.

MATERIALS AND METHODS

Seed source and sterilization treatment: Ripe fruits of *G. arborea* were collected from an eighteen-year-old tree growing in the experimental garden. Seeds were dissected and dried under the shade for 3-4 days. The air-dried seeds were washed thoroughly under running tap water followed by a 2 min treatment with a 5 % (v/v) aqueous solution of Laboline (Qualigens, India) and rinsed 5-6 times with distilled water. Further sterilization treatments were done under a laminar-air-flow chamber. The seeds were surface sterilized with 0.1 % (w/v) freshly prepared aqueous mercuric chloride (E-Merk, India) solution for 5 min and finally rinsed 4–5 times with autoclaved double distilled water.

Media and culture conditions: The surface-sterilized, seeds were aseptically cultured on to germination media. Three types of germination media were tested: full-strength MS (Murashige and Skoog 1962), half-strength MS and DDW (double distilled water) with 3 % sucrose (Qualigens, India) without any growth regulators and gelled with 0.7 % agar (Qualigens, India). The pH of the medium was adjusted to 5.8 prior to autoclaving at 121.4 °C and 105 kPa for 15 min. The cultures were incubated at 25 ± 2°C with 16 hr photoperiod of 35 - 50 mmol m^{-2} s^{-1} photon flux density provided by cool white fluorescent tubes (Philips, India) and 55-60 % relative humidity. Twenty-five seeds were used for each medium.

Two weeks old axenic seedlings served as the source of explants. After removal of the radicle and the primary shoots the cotyledonary nodes were inoculated into 250 ml conical flasks (Borosil, India) containing MS medium supplemented with various concentration of BA and TDZ singly or in combination with IAA or NAA. The pH of the medium was adjusted to 5.8 before gelling with 0.8 % agar. All cultures were maintained under similar conditions as described earlier for seed germination. Sub-culturing was done at 3-week intervals and done twice.

Rooting of micro shoots and hardening: In vitro regenerated shoots were carefully excised and cultured on half-strength MS basal medium 0.8 % agar for rooting. The medium was further supplemented with different concentrations of IBA (4.9, 9.8 & 14.7 μM) or NAA (5.7, 11.7 & 17.1 μM). After 5 week of cultures the rooted plantlets were carefully removed from the agar media, washed thoroughly under tap water and transferred to root trainers (Nivedita Traders, India) containing vermiculite. The transplanted shootlets were shifted to automated green house where the temperature was maintained around 28 ± 2°C with 85 - 90% humidity for hardening. After 2 weeks of hardening the plantlets were transferred to poly bags containing garden soil + FYM (farm yard manure) and kept under a shade-net house for another 2 weeks before planting in the field.

Scoring of data: Data on seed germination at weekly interval and, total number of shoots and its length from each cotyledonary node were determined 7 weeks after culture initiation. Similarly data on percent rooting, total root number and its length were determined 3 weeks after culture. Statistical analysis was carried out using analysis of variance (ANOVA) and least significant difference (LSD).

RESULTS

In vitro seed germination: Depending on the media, seeds showed germination between 3-20 days of inoculation. Quickest response was recorded in the DDW medium where germination initiated immediately after 3 days of culture; in rest of the media germination was observed after 5 days of culture. Seed germination was highest in the DDW medium where an average of 85.7% seeds germinated followed by 64.3% germination in half-strength MS medium and 57.1% in full-strength MS medium. Seed germination was pronounced during 2nd week and continued to germinate up to 3rd week (Fig. 1).

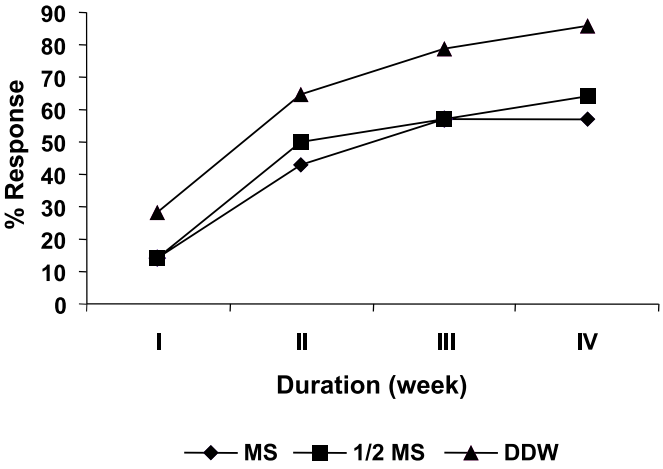

Fig. 1. Effect of different media on seed germination of *G. arborea*. MS: Full strength of MS medium, ½ MS: Half strength of MS medium, DDW: Double distilled water with 3% sucrose

Establishment of shoot cultures: The cotyledonary nodes responded in all the media combinations containing BA or TDZ either alone or in combinations with IAA or NAA (Table 1). Growth regulator supplemented MS basal medium significantly showed highest regenerative response than the medium without growth regulator. Shoot regeneration was observed within 3-5 days of its culturing in the growth regulator supplemented media (Fig. 2 a). Explants in growth regulator free media, showed regeneration after 8-10 days of culture. Growth and differentiation of shoots were always associated with little or profuse callusing and dedifferentiation at the cut end in contact of medium. Analysis of variance revealed that shoot number and shoot length were significantly affected by the concentration and type of growth regulator used (Table 1).

MS medium without any growth regulator showed a maximum response of 45 % with an average shoot number of 0.9 per explant. The addition of BA to the medium had positive effect on shoot formation. Percent response as well as the number of shoots produced per explant was high in MS + BA (4.4 μM) + IAA (1.4 μM) where about 80 % cultures showed response with an average shoot numbers of 15.5 per cotyledonary node. Also shoot growth was better in this medium composition. An average shoot length of 2.9 cm was recorded followed by 2.5 cm length in the medium with MS + BA (2.2 μM). Initially, shoot buds appeared as small protuberances (Fig. 2 b) subsequently these protuberances developed and elongated into slender shoots (Fig. 2 c). The frequency of shoot bud development and the number of shoots per explant increased with increasing concentration of BA from 2.2 μM to 4.4 μM. Further, the frequency of shoot bud production declined when the concentration of BA increased to 6.6 μM. It was observed that addition of IAA along with BA to the basal medium enhanced the response significantly. As it is seen from the data (Table 1) that when BA alone used as supplement to MS medium the maximum response was 70 % with an average shoot number of 5.1 per explant in the medium supplemented with 4.4 μM BA. Lower concentration (1.4 μM) of IAA favoured shoot growth along with BA, while higher concentration (2.8 μM) had inhibitory effect (Table 1). Addition of NAA with BA inhibited shoot growth and shoot development. A maximum of 55 % explants responded in the medium containing MS + 2.2 μM BA + 1.3 μM NAA with an average shoot numbers of 3.5 per explant. Higher concentration of BA and NAA

Fig. 2. Plant regeneration of *G. arborea* from cotyledonary node culture. (a) Shoot bud formation from cotyledonary node after 5 days in culture on MS + BA (4.4 μM) + IAA (1.4 μM), (b) Multiple shoot induction in cotyledonary node explant after 2 week in culture on medium MS + BA (4.4 μM) + IAA (1.4 μM), (c) Shoot elongation on the same medium after 5 week of culture, (d) *In vitro* shoot rooted on ½ MS + IBA (4.9 μM) + TIBA (2.0 μM) after 5 wk in culture, (e) An acclimatized plant in the poly pot (after 3 wk of transfer).

Table 1. Effect of BA, TDZ, IAA and NAA on cultured cotyledonary node explants of *Gmelina arborea* (10 explants per treatment, repeated twice; data scored after 7 weeks of culture)

MS + PGR (μM)				Days to shoot bud initiation	% Response	Average shoot numbers per cultured explant	Average shoot length per shoot (cm)
BA	**IAA**	**NAA**	**TDZ**				
0	0	0	0	8-10	45	0.9	1.1
2.2	0	0	0	4-6	65	3.5	2.5
4.4	0	0	0	4-6	70	5.1	2.1
6.6	0	0	0	4-6	70	4.6	1.7
LSD						2.6	1.2
F-value						4.5	1.6
P-value						0.009	0.2
2.2	1.4	0	0	3-4	70	7.5	2.3
4.4	1.4	0	0	3-4	80	15.5	2.9
6.6	1.4	0	0	3-4	60	6.3	1.6
LSD						4.5	1.3
F-value						7.57*	2.6
P-value						0.005	0.06
2.2	2.8	0	0	3-4	65	3.8	2.0
4.4	2.8	0	0	3-4	50	2.4	1.2
6.6	2.8	0	0	3-4	50	2.0	1.2
LSD						2.1	1.1
F-value						2.7	0.9
P-value						0.057	0.46
2.2	0	1.3	0	4-6	55	3.5	1.3
4.4	0	1.3	0	4-6	50	2.2	1.8
6.6	0	1.3	0	4-6	40	1.7	1.0
LSD						1.4	1.04
F-value						1.44	0.67
P-value						0.26	0.58
2.2	0	2.7	0	4-6	55	2.5	1.5
4.4	0	2.7	0	4-6	55	2.0	1.2
6.6	0	2.7	0	4-6	40	1.7	1.0
LSD						1.13	0.83
F-value						0.39	0.39
P-value						0.68	0.68
0	0	0	2.3	4-5	60	3.0	1.2
0	0	0	4.5	4-5	55	2.2	1.0
0	0	0	6.8	4-5	40	1.3	0.7
LSD						1.1	0.8
F-value						1.58	0.54
P-value						0.24	0.59

had inhibitory effect on shoot production. In such cultures explants showed profuse callusing with fewer shoot (Table 1).

Cultures maintained in the medium with MS + TDZ responded poorly in all the concentration tested. Maximum response was recorded in MS + 2.3 μM TDZ where about 60 % nodes responded with an average shoot numbers of 3.0 per explant. As the concentration increased there was reduction in the shoot numbers and also shoot growth. Most of the cultures showed callusing symptoms immediately after 1 week of culture. Multiple shoots obtained in TDZ supplemented medium where stunted and no appreciable length was recorded (Table 1).

In vitro rooting of microshoots: The rooting responses of shoots on different media, which included rooting percentage, days required for root initiation, average root number and average root length over a period of five weeks have been presented in table 2. *In vitro* rooting in the shootlets excised from the cultured cotyledonary nodes was achieved on a half strength MS basal medium enriched with growth regulator. Depending on the media composition and concentration, roots initiated between 8 to 14 days after culturing in the rooting medium. Medium devoid of auxin showed maximum response of 53.3 % with an average root number and root length of 1.3 and 1.2 respectively per plantlet. Rooting was better in the culture which had combination of ½ MS + 4.9 μM IBA + 2.0 μM TIBA where about 66.6 % cultures responded for rooting with an average root number of 3.2 roots per plantlet and an average root length of 2.2 cm was recorded (Fig. 2 d). It was observed that always root formation proceeded with formation of callus at the base. The intensity of callus was high in the medium supplemented with higher concentration of auxins where the number of roots produced per shoot and root growth was poor (Table 2). In order to suppress the callus growth, TIBA was added to the medium and callus growth was found to be restricted to some extend. Among two auxin tested, IBA found to be superior than IAA in terms of root biomass (Table 2).

Rooted plantlets were taken out from the culture tubes, washed thoroughly to remove any remains of medium and planted in small plastic pots containing pre-soaked vermiculite. The plantlets were maintained inside a mist-chamber for a period of 3 weeks (Fig. 2 e) and were subsequently transferred to the field. About 70 % of the plantlets survived in the field.

Table 2. Effect of IAA, IBA and TIBA on rooting response of in vitro shoots reared from cotyledonary node explants of *Gmelina arborea* (10 explants per treatment, repeated twice; data scored after 5 wk of culture)

Growth regulators augmented with ½ MS basal medium (μM)	Days to root initiation	Percent response	Average root numbers per shoot	Root length per shoot (cm)
IAA				
0	12 – 14	53.3	1.3	1.2
5.7	8 – 10	60.0	2.3	1.8
11.4	8 – 10	56.6	1.5	1.1
17.1	8 – 10	53.3	1.2	0.9
LSD			1.198	1.017
P-value			0.407	0.396
F-value			0.992	1.016
IBA				
4.9	8 – 10	66.6	3.1	1.9
9.8	8 – 10	53.3	1.9	1.4
14.7	8 – 10	50.0	1.1	0.9
LSD			1.121	0.944
P-value			0.033	0.213
F-value			3.231	1.648
IAA + TIBA				
5.7 + 2.0	8 – 10	50.0	2.3	2.4
11.4 + 2.0	8 – 10	66.6	1.4	1.6
17.1 + 2.0	8 – 10	56.6	1.4	1.5
LSD			0.941	0.935
P-value			0.339	0.431
F-value			1.123	0.866
IBA + TIBA				
4.9 + 2.0	8 – 10	66.6	3.2	2.2
9.8 + 2.0	8 – 10	56.6	1.9	1.5
14.7 + 2.0	8 – 10	50.0	1.2	1.2
LSD			0.805	0.030
P-value			0.256	0.140
F-value			4.207	2.114

DISCUSSION

MS medium has frequently been used successfully for shoot induction in tree tissue culture studies (Dunston and Thorpe 1986). The superiority of MS over other salt formulations has been demonstrated in other trees such as *Prosopis cineraria* (Shekawat *et al.* 1993), *Swartzia madagascarensis* (Berger and Schaffner 1995), *Bauhinia vahlii* (Upreti and Dhar 1996) including *Gmelina arborea* (Thirunavoukkarasu and Debata 1998). MS basal medium was used in the present studies. For shoot induction growth regulators such as BA, TDZ, IAA and NAA were tested. The concentration and the type of growth regulators used had profound influence on shoot development from cotyledonary nodes of *G. arborea*. MS medium supplemented with BA showed highest regenerative response than TDZ supplemented medium. BA is reported to have favoured shoot proliferation from cotyledonary nodes of several tree species such as *Acacia tortilis* (Nandwani 1995), *Dalbergia sissoo* (Pradhan *et al.* 1998), *Terminalia chebula* (Shyamkumar *et al.* 2003). In an attempt to obtain shoot multiplication for *in vitro* propagation of *Swartzia madagascarensis* 37 shoots/explants could be induced in MS medium containing 2.2 μM BAP (Berger and Schaffner 1995).

In the present study cotyledonary nodes of *G. arborea* showed significantly higher response in the medium with the combination of BA + IAA. The quality of shoots and the overall growth response in terms of average shoot length was also better in this growth regulator combination. A comparatively lower response was recorded when BA was used singly in the medium. A review of literatures indicates that addition of either of IAA or NAA in the culture medium improved the response in a number of species in terms of shoot growth. Ramirez-Malagon *et al.* (2001) reported that axillary shoots of *Spathiphyllum floribundam* when cultured on media with BA supplement alone a limited proliferation of explants with a maximum of average of 1.8 shoots per cultured explant was observed, while addition of IAA produced an average shoots of 11.6 per explant. We observed that when BA was used as sole supplement in MS medium, maximum shoot numbers recorded was 5.1. Whereas, addition of IAA (1.4 μM) to the medium an average shoot numbers of 15.5 per explant could be achieved. This is in corroboration with the observation reported in *Hovenia dulcis* nodal culture (Echererigeray *et al*., 1998). When a combination of BA + NAA tested, the frequency of shoot formation was reduced, as reported in the case of *Tylophora indica* (Faisal and Anis 2003). Contrary to this, Bandyopadhyay *et al.* (1999) reported that hypocotyl explants of *Eucalyptus nitens* and *E. globulus* showed high regenerative capacity when they were cultured on to MS basal medium supplemented with BA and NAA.

Regenerated shoots could be rooted on half-strength MS basal medium supplemented with or without auxins. We observed relatively better rooting when the shoots were cultured in the medium supplemented with low concentration of auxins. Although rooting occurred in the medium devoid of any growth regulators, percent rooting and root biomass was inferior to the medium supplemented with auxin. Formation of roots in the medium without auxin may be attributed to the high level of endogenous auxin in the shoots. A range of concentrations of IAA and IBA tested, both these auxins were effective in the lower concentration. Higher concentration of these auxins resulted in callusing and reduction in root number as well as poor root growth. Similar observation is reported in *Khaya senegalensis* (Danthu *et al.* 2003) where rooting of *in vitro* shoots could be achieved in 5.2 μM IBA. Requirement of auxin for root induction has been reported in a number of tree species such as *Dalbergia latifolia* (Raghava Swamy *et al.* 1992), *Citrus aurantifolia* (Al-Bahrany 2002) and *Achras sapota* (Purohit and Singhvi 1998). Between two auxin tested for rooting, IBA proved to be superior to IAA. IBA has been widely used as a root-inducing hormone in difficult-to-root plants both under *in vitro* and *in vivo* conditions (Minocha 1987, Purohit & Singhvi 1998). We observed excessive callusing in the medium supplemented with IBA, addition of TIBA reduced callusing to some extent. TIBA reported to inhibit transport of auxin from shoot to the root zone (Kumar *et al.* 2005).

The shoot regeneration potential observed in the present study was much higher than the earlier reports, where the maximum shoot numbers achieved were less

than ten shoots per explant (Kannan and Jasrai, 1996; Thirunavoukkarasu and Debata, 1998; Valverde-Cerdas, *et al*., 2004). This study provided a simple *in vitro* method for the propagation of *G. arborea* from cotyledonary nodes. Where maximum of 15.5 shoots were produced in MS medium with BA (4.4 μM) + IAA (1.4 μM). Although, the genetic fidelity of the micropropagated plants can be ensured only by using explants from mature material but the seedling material frequently used in tree propagation programme form the basis of development of suitable nutrient media and methods for their future use with mature material.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. B.K. Mishra, Director, Institute of Minerals & Materials Technology, Bhubaneswar for facilities and encouragement. We also thank Shri K.K. Rao, Scientist, RPBD Department for statistical analysis of the data. The Department of ISM, Ministry of Health and Family Welfare is duly acknowledged for sponsoring this project.

REFERENCES

Al-Bahrany, A.M. (2002). Effect of phytohormones on *in vitro* shoot multiplication and rooting of lime *Citrus aurantifolia* (Christm.) Swing. *Scientia Hortic*. **95:** 285-295.

Arya, R.S. and Haque, M.S. (1982). Grafting and budding in yemane *Gmelina arborea* Roxb. *Ind. For*. **108:** 497-500.

Bandyopadhyay, S., Cane, K., Rasmussen, G. and Hamill, J.D. (1999). Efficient plant regeneration from seedling explants of two commercially important temperate *Eucalyptus* species – *Eucalyptus nitens* and *E. globules*. *Plant Sci.* **140:** 189-198.

Berger, K. and Schaffner, W. (1995). *In vitro* propagation of the leguminous tree *Swartzia madagascariensis*. *Plant Cell Tissue Org. Cult.* **40:** 289-291.

Chopra, R.N., Nayar, S.L. and Chopra, I.C. (1956). Glossary of Indian Medicinal Plants. CSIR, New Delhi.

Danthu, P., Diaite-Sanogo, P., Sagna, M., Sagna, P. and Dia-Gssama, Y.K. (2003). Micropropagation of *Khaya senegalensis*, an African mahogany from dry tropical zones. *J. Trop. For. Sci.* **15:** 164-175.

Dunstan, D.T. and Thorpe, T.A. (1986). Regeneration in forest trees. In: I.K. Vasil (ed.), Cell Culture and Somatic Cell Genetics of Plant. Vol. 3, pp. 223-241. Academic Press, New York.

Dvorak, W.S. (2003). World view of *Gmelina arborea*: Opportunities and challenges. In: W.S. Dvorak, G.R. Hodge, W.C. Wood-bridge and J.L. Romero (eds), Proc. Recent Advances in *Gmelina arborea*. CD-ROM. CAM-CORE. North Carolina State University, Raleigh, NC, USA.

Echeverrigaray, S., Mossi, A.J. and Munari, F. (1998). Micropropagation of raisin tree (*Hovenia dulcis* Thunb.) through axillary bud culture. *J. Plant Biochem. Biotechnol.* **7:** 99-102.

Faisal, M. and Anis, M. (2003). Rapid mass propagation of *Tylophora indica* Merrill via leaf callus culture. *Plant Cell Tissue Org. Cult.***78:** 125-129.

Joshi, K., Singh, P. and Pardasani, R.J. (1977). Chemical components of the root of *Tectona grandis* and *Gmelina arborea*. *Planta Medi*. **32:** 71-75.

Kannan V.R. and Jasrai Y.T.(1997). Micropropagation of Gmelina arborea. *Plant Cell Tissue Org. Cult.* **46:** 269-271

Kumar, V., Gururaj, H.B., Prasad, B.C.N., Giridhar, P. and Ravishankar, G.A. (2005). Direct shoot organogenesis on shoot apex from seedling explants of *Capsicum annuum* L. *Scientia Hortic.* **106:** 237-246.

Little, E.L. (1983). Common Fuelwood Crops: A Handbook for Their Identification. McClain Printing Co., Parsons, WV.

Minocha, S.C. (1987). Plant growth regulators and morphogenesis in cell and tissue culture of forest trees. In: J.M. Bonga and D.J. Durzan (eds.), Cell and Tissue Culture in Forestry, pp. 56. Martinus Nijhoff Publishers.

Murashige T. and Skoog F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant.* **15:** 473-497.

Nandwani, D. (1995). *In vitro* micropropagation of a tree legume adapted to arid lands *Acacia tortilis* subsp. *raddiana*. *Ann. Sci. For.* **52:** 183-189.

Pradhan, C., Kar, S., Pattnaik, S. and Chand, P.K. (1998). Propagation of *Dalbergia sissoo* Roxb. through *in vitro*

shoot proliferation from cotyledonary nodes. *Plant Cell Rep.* **18:** 122-126.

Purohit, S.D. and Singhvi, A. (1998). Micropropagation of *Achras sapota* through enhanced axillary branching. *Scientia Hortic.* **76:** 219-229

Raghav Swamy, B.V., Himabindu, K. and Sita, L.G. (1992). *In vitro* micropropagation of elite rosewood (*Dalbergia latifolia* Roxb). *Plant Cell Rep.* **11:** 126-131.

Ramirez-Malagon, R., Borodanenko, A., Barrera-Guerra J.L. and Ochoa-Alejo, N. (2001). Shoot number and shoot size as affected by growth regulators in *in vitro* cultures of *Spathyphyllum floribundum* L. *Scientia. Hortic.* **89:** 227-236.

Rao, D.V. and Rao, E.V. (1970). Chemical components of leaves of *Gmelina arborea*. *Ind. J. Pharm.* **32:** 140-141.

Roy, K.S. (1995). In vitro micropropagation techniques of high quality planting stock of some timber trees. *Plant Tiss. Cult.* **5:** 88.

Satyanarayana, P. and Rao, P.K. (1986). Arborene and 7-oxodihydrogmetinol-Two new ketolignans from *Gmelina arborea. J. Nat. Prod.* **49:** 1061-1064.

Shekhawat, N.S., Rathore, T.S. and Singh, R.P. (1993). Factors affecting *in vitro* clonal propagation of *Prosopis cineraria. Plant Growth Regul.* **12:** 273-280.

Shyamkumar, B., Anjaneyulu, C. and Giri, C.C. (2003). Multiple shoot induction from cotyledonary node explants of *Terminalia chebula*. *Biologia Plant.* **47:** 585-588.

Subramanian, K.N., Singh, B.G. and Jayachandran, C.K. (1992). Propagation of *Gmelina arborea* Roxb. by bud sprouts. In: K.K Reddy (ed.). Proc. Vegetative Propagation and Biotechnologies for Tree Improvement, pp17-19. Natraj Publishers, Dehradun.

Surendran, C.(1990). Vegetative propagation in *Gmelina arborea* Roxb. through single nodded cuttings. *Ind. J. Forestry.* **13:** 162-164.

Thirunavoukkarasu, M. and Debata, B.K. (1998). Micropropagation of *Gmelina arborea* Roxb. through axillary bud culture. *Ind. J. Plant Physiol.* **3:** 82-85.

Upreti, J. and Dhar, U. (1996). Micropropagation of *Bauhinia vahlii* Wight & Arnott – A Leguminous Liana. *Plant Cell Rep.* **16:** 250-254.

Valverde-Cerdas, L., Alvarado, L. and Hine, A. (2004). Micropropagation of clones from controlled crosses of *Gmelina arborea* in Costa Rica. *New For.* **28:** 187-194.