Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PROTOCOL FOR PLANTLET REGENERATION VIA ENHANCED SHOOT PROLIFERATION IN *MURRAYA KOENIGII* (L.) SPRENG.

NISHA KHATIK AND RAMESH JOSHI*

Plant Biotechnology Laboratory, Department of Botany, Government College Ajmer, Ajmer, Rajasthan 305001, India.

ABSTRACT

An efficient protocol for high frequency regeneration of plantlets from epicotyl and cotyledonary node explants of an important medicinal plant *Murraya koenigii* was developed. Explants were obtained from 60 days old axenic seedlings of *Murraya koenigii* and cultured on Murashige and Skoog (MS) medium supplemented with plant growth regulators to ascertain the suitable explants and media composition for mass production. Multiple shoot induction was achieved on Murashige and Skoog (MS) basal medium augmented with different concentrations of 6-benzyleaminopurine (BAP) 2.78 to 17.38 µM, Kinetin (Kin.) 0.62 to 11.21 µM and Indole-3-acetic acid (IAA) 0.29 to 5.12 µM. Percentage response of epicotyl explants was 95.2±0.07 which was significantly higher than the response of cotyledonary node (89.4±0.34%) explant. The 40-45 mm elongated shoots were cultured to in MS basal medium augmented with different concentrations of indole-3-butyric acid (IBA). The maximum percentage, 93.4±2.30 of rooting was achieved on MS basal medium containing 9.98 µM IBA. The plantlets were hardened prior to field transfer. Ninety percent hardened plantlets were successfully survived under natural conditions.

KEYWORDS: ***Murraya koenigii,*** **Seedling, Cotyledonary node, Epicotyl, Micropropagation, Rutaceae**

*Corresponding author

RAMESH JOSHI
Plant Biotechnology Laboratory, Department of Botany, Government College Ajmer, Ajmer, Rajasthan 305001, India.

INTRODUCTION

Curry leaf is a small tree with dark gray bark and compound leaves[18]. In India, this plant is commonly found in the outer Himalayas, from the Ravi eastwards, ascending to 5,000 feet, in Assam, Chittagong, Upper and Lower Burma. Almost every part of this plant has a strong characteristic odor. Its leaves are slightly pungent, bitter, and acidulous in taste. Fresh and dried leaves are used extensively as a flavoring agent in many Indian culinary practices. The fresh leaves of this tree possess antimicrobial, topoisomerases and mosquitocidal inhibition and antioxidant properties[38,21,31,14]. Recent reports on successful plant regeneration from juvenile epicotyl explants of various woody legumes, including *Tetrapleura* tetraptera, *Parkia biglobosa, Albizia odoratissima* and *Piliostigma thonningii* [6,2,35,5]. As cotyledonary node explants have already been recommended, as an excellent tissue, in several studies conducted in other tree [32,39,3]. In general cotyledonary node explant found are second most effectual explant in obtaining good quality of shoots for large scale multiplication and conservation. Present study aims at the application of tissue culture techniques to develop an effective protocol for high frequency shoots regeneration in *Murraya koenigii* (L.) Spreng from epicotyl (EP) and cotyledonary node (CN) explants.

MATERIALS AND METHODS

(i) Explant preparation

Fruits of *Murraya koenigii* were obtained in the month of June to the end of July from surrounding area of Ajmer, Rajasthan, India. Seeds from mature fruits were carefully taken out by removing the pulp of fruits with the help of forceps & scalpel and then washed with liquid detergent (Teepol; Qualigen, India) for 2min. and then treated with a 0.1% solution of Bavistin fungicide (BASF, India) for about 5 min. to remove fungal contaminants from the explants. The seeds were surface sterilized with 0.1% aqueous HgCl2 solution for 5-6 min. and then rinsed 4-5 times with autoclaved distilled water.

(ii) Nutrient media and culture conditions

The nutrient medium consisted of Murashige and Skoog (MS) basal medium supplemented with sucrose (3% w/v). Disinfected seeds were germinated in 200 ml screw-capped glass jars containing 40 ml seed germinating half strength MS basal medium devoid of plant growth regulators[27]. Epicotyl and Cotyledonary node were excised from 60 days old seedling as explants. *In vitro* shoots were induced on MS medium supplemented with different plant growth regulators such as 6-benzylaminopurine (BAP, 2.78 to 17.38 µM), Kinetin (Kin, 0.62 to 9.21 µM), and Indole-3-acetic acid (IAA, 0.29 to 5.12 µM) alone or in combination to MS basal medium. The *in vitro* raised shoots (40-45 mm) were excised and individually transferred on MS medium containing different concentration of indole-3-butyric acid (IBA, 2.27 to 14.79 µM) for rooting. Media were solidified by adding 0.8% agar powder (Qualigen, India). The pH of media was adjusted at 5.8 and was autoclaved at temperature 121°C and 15 psi pressure for 15-20 minutes. All the cultures were incubated in a culture room maintained at 25 ± 2°C under 16/8 h light/dark cycle, 45 µM m−2 s−1 irradiance level provided by cool white fluorescent tubes. Each treatment consisted of 10 explants and was repeated thrice.

(iii) Acclimatization and field transfer

In vitro developed plantlets with 40-45 mm shoot length and strong taproot were washed with running tap water and were transferred into 200 ml jars 1/3 filled with a pasteurized mixture of vermiculite, perlite and peat moss in equal ratio. The plantlets in the screw capped jars were kept under a hardening unit for one week and then the screw caps were removed. They were later gradually transferred to the low humidity and high light intensity zone of the hardening unit in the interval of one week. The plantlets were finally transferred to poly bags and exposed to field conditions.

(iv) Statistical analysis

Experiments were set up in completely randomized design with 10 replicates per treatment and each experiment was repeated thrice. Mean values were subjected to analysis of variance (ANOVA) and statistically significant ($P < 0.05$) means were determined by new Duncan's Multiple- range test [15].

RESULTS

(i) In vitro seed germination and explant preparation

The surface sterilized seeds were inoculated on half strength Murashige and Skoog (MS) basal medium for germination. *In vitro* cultured seeds showed 89% germination after 1 to 2 weeks of inoculation and attain a height of 6 to 7 cm in 4 to 5 weeks. EP and CN were excised from 4-5 week old *in vitro* raised seedling and used as explants

(ii) Multiple shoots regeneration

The explants were inoculated on MS basal medium augmented with or without plant growth regulators. Observations were recorded on control, MS without PGRs, medium 59.2±0.04 and 53.4±0.30 percentage of shoots were achieved from epicotyl and cotyledonary node explants respectively. Addition of plant growth hormones to the medium had a positive effect on shoot formation from both the explants. However, the frequency of responding explants and number of shoots per explants varied in different concentrations of PGRs and type of the explants. The multiple shoots induction was achieved on MS basal medium supplemented with different concentrations of BAP 2.78 to 17.38 µM, Kinetin 0.62 to 9.21 µM, and IAA 0.29 to 5.12 µM alone or in combination to MS basal medium (Table 1). Highest, 95.2±0.07 percent cotyledonary node explants responded for induction of 7.8±0.09 shoots per explants (Fig 1a) and 89.4±0.37 percent response was observed in cotyledonary node explants for induction of 6.7±0.09 shoots per explant (Fig 1b) on MS basal medium supplemented with BAP 12.4µM, Kinetin 9.21µM and IAA 2.87µM

.

Table 1

Effect of different concentrations of growth regulators in MS basal medium on multiple shoot induction from Epicotyl and Cotyledonary node explants of Murraya koenigii.

PGRs			Epicotyl			Cotyledonary node		
BAP (µM)	Kinetin (µM)	IAA (µM)	Explant response (%) for shoot initiation (Mean±S.D.)	No. of shoots per explant (Mean±S.D)	Length of shoots in mm (Mean±S.D.)	Explant response (%)for shoot initiation (Mean ± S.D.)	No. of shoots per explant (Mean±S.D	Length of shoots in mm (Mean±S.D.)
0.00	0.00	0.00	59.2±0.04a	2.3±0.04df	9.8±0.05a	53.4±0.30cc	2.1±0.02f	4.8±0.07ca
4.27	0.00	0.00	75.6±0.40ca	4.4±0.08ad	14.0±0.04e	65.4±1.14a	2.8±0.06ef	9.8±0.05ac
8.52	0.00	0.00	81.0±0.54cd	5.2±0.07c	21.8±0.03g	73.6±0.23cd	3.5±0.04d	14.0±0.02b
12.54	0.00	0.00	85.9±0.52aa	5.7±0.05g	23.1±0.02k	81.0±0.54bd	4.5±0.05ad	18.0±0.04h
17.38	0.00	0.00	82.4±0.36c	4.3±0.02ca	21.8±0.03ij	74.4±1.12dc	4.2±0.43gh	15.6±0.09f
0.00	4.62	0.00	58.2±0.03g	2.8±0.06ac	11.2±0.02gh	59.8±1.78gh	2.0±0.08a	5.6±0.04g
0.00	6.89	0.00	63.4±0.30bb	3.5±0.24gh	12.4±0.06d	60.4±1.14ij	3.4±0.04cd	9.8±0.05ac
0.00	9.21	0.00	78.6±0.04d	4.2±0.07cc	17.3±0.09ac	73.6±0.23cdk	3.9±0.09cc	12.4±0.06ij
0.00	11.34	0.00	75.6±0.40fg	3.5±0.24gh	14.0±0.04e	63.4±0.04d	3.5±0.04d	11.2±0.02aa
12.54	9.21	0.00	81.0±0.54cd	5.8±0.03d	15.6±0.09c	80.6±2.40ad	4.5±0.05ad	16.1±0.34bd
12.54	9.21	0.58	85.8±0.04h	6.1±0.04ef	18.3±0.56b	84.6±0.63i	4.9±0.07ij	19.1±0.09jk
12.54	9.21	1.73	91.0±0.09ij	6.9±0.05jk	25.9±0.21cd	85.8± 0.83f	5.6±0.02fd	21.6±0.17ef
12.54	9.21	2.87	95.2±0.07bd	7.8±0.09fd	30.0±0.05bd	89.4±0.37h	6.7±0.09aa	28.4±0.07cd
12.54	9.21	3.92	89.2±0.13ef	6.7±0.02cb	28.4±0.07ef	86.2±1.14e	6.2±0.03b	26.9±006gh
12.54	9.21	5.12	87.4±0.34cf	5.9±0.03aa	26.9±006ad	84.6±0.63i	5.9±0.05e	21.6±0.17ef

P < 0.05; Each value represents the mean ± Standard deviation (SD) of ten replicates per treatment in three repeated experiments, PGRs plant growth regulators, BAP 6- benzylaminopurine, IAA Indole-3-acetic acid

(iii) Shoot multiplication

Regenerated *in vitro* shoots were excised from explant and sub- cultured to the MS medium augmented with combinations of BAP, Kinetin and IAA in different concentrations (Table-2). Compact shoot clumps were formed in a period of 3-4 week. Multiplication of shoot clumps varied in different explants and on various concentrations of BAP, Kinetin and IAA in MS basal medium. An average 4.1±0.06 and 3.9±0.08 fold shoot multiplication was achieved in EP and CN explants respectively (Fig. 1c).

Table 2

Effect of different concentrations of BAP and Kinetin with ADS (Adenine sulphate) in MS basal medium on shoot multiplication from Epicotyl and Cotyledonary node explants of Murraya koenigii.

PGRs			Epicotyl	Cotyledonary node
BAP (µM)	Kinetin (µM)	IAA (µM)	Multiplication Rate (Mean ± S.D.)	Multiplication Rate (Mean ± S.D.)
2.78	0.62	0.00	2.4±0.04bc	1.8±0.09df
4.27	2.54	0.29	3.2±0.03a	2.4±0.04cc
8.52	4.62	0.58	4.1±0.06d	3.9±0.08ad
12.54	6.89	1.73	3.6±0.02f	2.2±0.03gh
17.38	9.21	2.87	2.4±0.04bc	2.0±0.06ij

$P < 0.05$; Each value represents the mean ± Standard deviation (SD) of ten replicates per treatment in three repeated experiments, PGRs plant growth regulators, BAP 6- benzylaminopurine, IAA Indole-3-acetic acid

(iv) Rooting

The *in vitro* raised shoots recovered from all the explants when attained a length of 40-45 mm. were transferred to MS basal medium augmented with or without plant growth regulators for root induction. In control medium 19.0±1.00 and 17.2±0.83 percentage of rooting was achieved from epicotyl and cotyledonary node explants respectively. IBA at different concentrations (2.27 to 14.79 µM) showed different responses in terms of percentage and growth of roots *in vitro* (Table 3).The maximum 93.4 ± 2.30 percent rooting was achieved from shoots of epicotyl origin on MS medium supplemented with 9.98 µM IBA whereas on the same medium 88.0 ± 1.00 percent rooting was achieved from shoots of cotyledonary node origin (Fig. 1d).

(v) Establishment of plantlets

In vitro plantlets were hardened in small earthen pots containing a mixture of Soil Rite (peat moss: perlite: vermiculite; 1: 1: 1) at 70-80% relative humidity and temperature gradient of 28-36°C under green house conditions for 21 days. Survival rate was 90% in hardened plantlets (Fig.1 e). These plants were then transferred to field conditions (Fig. 1f).

Table 3

Effect of different concentrations of IBA in MS medium on rooting of in vitro adventitious shoots of Murraya koenigii from Epicotyl and Cotyledonary node explants.

IBA (µM)	Rooting (%)	
	Epicotyl (Mean ± SD)	Cotyledonary node (Mean ± SD)
0.0	19.0±1.00a	17.2±0.83cd
2.47	42.6±1.14d	39.8±0.73gh
4.92	65.2±2.40c	60.2±2.40ef
7.51	86.2±0.45ef	71.2±0.13ij
9.98	93.4±2.30b	88.0±1.00df
12.25	89.2±0.83j	83.2±1.13ab
14.79	86.2±0.45ef	81.2±0.21cc

$P < 0.01$; Each value represents the mean ± Standard deviation (SD) often replicates per treatment in three repeated experiments; PGRs plant growth regulators, IBA indole-3-butyric acid

Figure. 1a-f

High frequency plantlet regeneration from Epicotyl and Cotyledonry node explants of Murraya koenigii: (a) Shoot regeneration from Epicotyl, (b) Shoot regeneration from Cotyledonry node explant, (c) Shoot multiplication, (d)Rooting, (e) Six week-old tap rooted plantlets prior to hardening, (f) Hardened field growing plants of M. koenigii.

DISCUSSION

The shoot bud formation efficiency of cultured explants showed varied response and seems to be dependent more precisely on the explant type as well as on the plant growth regulator treatments. The results reported herein indicate that besides culture media composition and explant type, there is a marked influence of genotype in the success of *in vitro* culture[8]. In the present study, addition of BAP in combinations with Kinetin and IAA to the regeneration medium used for shoots formation. Similar observations were reported in several other plants such as *Feronia limonia* and *Aegle marmelos*[16,10]. This observation is in agreement with the previously published works demonstrating BA as the most successful cytokinin for shoot organogenesis in several other systems including *Bacopa monnieri*, *Holarrhena pubescens, Cynodon dactylon, Salvia officinalis* and *Scopolia parviflora* [43,40,47,41,19]. It is common to observe a relationship between BA concentrations and shoot number and shoot size[9]. In present investigation higher concentration of BAP 12.54 µM, kinetin 9.21 µM and IAA 2.87 µM in MS basal medium favored highest shoot induction

and the lower concentrations of BAP 8.52 µM, Kinetin 4.62 µM and IAA 0.58 µM favored subsequent shoot multiplication. Experiments involving combination of auxin and cytokinins together showed a significant increment in the rate of shoot production from each explants tried. Cytokinin can be used alone or in combination with a low concentration of auxins [12,45,46].The promoting effect of auxin and cytokinin combinations on organogenic differentiation has been well documented in several systems [11,42,20,44,30,33]. Micropropagation protocols are successful only when the rate of survival of the *in vitro* regenerated plantlet is very high after transplantation. This in turn depends mainly on the development of a proper root system. Concentration and type of auxin in the medium were found to be the critical factor in producing healthy roots. The rooting methods in our study revealed that the presence of an exogenous auxin was vital for *in vitro* root induction of micro-shoots and IBA has been found to be the most effective auxin for *in vitro* rooting in *Murraya koenigii* shoots. The superior effects of IBA on the root development may be due to several factors such as its preferential uptake, transport and stability over other auxins and subsequent gene activation [22]. The present findings also showed similarities with *Calotropis gigantean, Capsicum annum* and *Prunus* sp., as they obtained root only with IBA[37,36,1,23]. IBA is considered as the most effective growth regulator for induction of roots in legumes[29]. IBA stimulated rooting was observed in *Vigna radiata, Aegle marmelos*, *Clitoria ternatea* and *Cotinus coggygria*[17,28,7,26]. Hardening and acclimatization procedures for establishment of micropropagated plantlets were also developed for many species such as *Salvadora persica, Peganum harmala*[25,13]. Several reports are available for many plant species such as *Celastrus Paniculatus, Dalbergia latifolia* and *Dendrocalamus asper* in which soil, sand and composed in the ratio of 1:1:1 was used for acclimatization of micropropagated plants[24,34,4]. In present investigation the plants were hardened in a mixture of perlite, vermiculite and peat moss in equal ratio. The *in vitro* plantlets developed during the study program were successfully hardened and transferred to the field where 90% plants were found healthy.

CONCLUSION

In the present study, a protocol has been worked out on the high frequency of shoot regeneration from Epicotyl and Cotyledonry node from axenic seedling explant of *Murraya koenigii,* which can be utilized for enhanced production of *M. koenigii* and its other close relatives of family Rutaceae.

ACKNOWLEDGMENT

The authors are thankful to the University Grants Commission (UGC), New Delhi for the financial support.

REFERENCES

1. Agrawal, S., Chandra, N. and Kothari, S. L. Plant regeneration in tissue cultures of pepper (*Capsicum annuum* L. cv. *mathania*). Plant Cell, Tissue Organ Culture 16: 47-55, (1989)
2. Amoo, S.O., and Ayisire, B.E. Induction of callus and somatic embryogenesis from cotyledon explants of *Parkia biglobosa* (Jacq.) Benth. Afr J Biotech. 4: 68-71, (2005).
3. Anis, M., Husain, M.K., and Shahzad, A. *In vitro* plantlet regeneration of Pterocarpus marsupium Roxb., an endangered leguminous tree. Curr Sci 88:861–863, (2005)
4. Arya, I.D., and Arya, S. *In vitro* culture and establishment of exotic bamboo *Dendro calamus asper.* Indian Journal of Experimental Biology; 35: 1252-1255, (1997).
5. Ayisire, B.E., Akinro, L.A., and Amoo, S.O. Seed germination and *in vitro* propagation *of Piliostigma thonningii*- an important

medicinal plant. Afr J Biotech. 8: 401-404, (2009).
6. Ayisire, B.E., and Amoo, S.O. Callus production and plant regeneration from hypocotyle and cotyledon explants of *Tetrapleura tetraptera* (Schum and Thonn.). Taub Environtropica. 1: 442-443, (2004).
7. Barik, D.P., Naik, S.K., Mudgal, A., and Chand, P.K. Rapid plant regeneration through *in vitro* axillary shoot proliferation of butterfly pea (*Clitoria ternatea* L.)-a twinning legume. In Vitro Cell Dev Biol. 43: 144-148, (2007).
8. Bordon, Y., Guardiola, J.L., and Garia-Luis, A. Genotype affects the morphogenic response *in vitro* of epicotyl segments of *Citrus* rootstocks. Annals of Botany, London, v.86, p.159-166, (2000).
9. Chevreau, B.T., and Arnaud, Y. Micropropagation of Pear (*Pyrus commuins* L.), In Y.P.S. Bajaj (eds), Biotechnology in Agriculture and Forestry, (Vol. 18), High-Tech and Micropropagation II, Springer-Verlag Berlin Heidelberg., (1992).
10. Das, R., Hasan, M.F., Rahaman, M.S., Rashid, M.H., and Rahman, M. Study on *In vitro* propagation through multiple shoot proliferation in wood apple (*Aegle marmelos* L.). Int. J. Sustain. Crop Prod, 3(6), 16-20, (2008).
11. Dimech, A.M., Cross, R., Ford, R., and Taylor, P.W. Micropropagation of Gymea Lily (*Doryanthes excels* Correˆa) from New South Wales, Australia. Plant Cell Tiss Organ Cult. 88: 157-165, (2007).
12. Dong, J-Z., Jia, S-R. High efficiency plant regeneration from cotyledons of watermelon (*Citrullus vulgaris* Schrad). Plant Cell Rep 9, 559–662, (1991).
13. Goel, N., Singh, N., and Saini, R. Efficient *in vitro* multiplication of Syrian Rue (*Peganum harmala* L.) Using 6-benzylaminopurine pre-conditioned seedling explants. Nature and Science, 7:129-134, (2009).
14. Gomes, L., Leitao, F., Coelho, A.V., and Boas, L.V. Phenolic compounds and antioxidant activity of *Olea europaea* L, fruits and leaves. Food Sciences and Technology International .12: 385-395, (2006).
15. Gomez, K.A., and Gomez, K.A. Statistical procedure for agricultural research with emphasis of Rice. Los Bans. Philippines International Rice Research, 50, 155-158, (1976).
16. Hiregoudar, L.V., Murthy, H.N., Hema, B.P., Haha,. E.J., and Paek, Y. Multiple shoot induction and plant regeneration of *Feronia limonia* (L.) Swingle. Scientia Horticulturae, 98(4): 357-364, (2003)
17. Husan, S.A.H., and Siddquai, S.A. Studies on Morphogenetic potentials of stem explants of *Vigna radiata* (L.) Wilczek. Bull J App Pure Sci. 19: 73-75, (2006).
18. Jayaweera, D.M.A. Medicinal Plants (Indigenous and Exotic) Used in Ceylon, The National Science Council of Sri Lanka, Colombo, (1982).
19. Kim, Y.D., Min, J.Y., Kim, W.J., Kang, Y.M., Moon, H.S., Lee, C.H., Prasad, D.T., Choi, M.S. High frequency plant regeneration and accumulation of tropane alkaloids in regenerated plants of *Scopolia parviflora*. DOI 10.1007/s11627-007-9073-2, (2009).
20. Koroch, A., Juliani, H.R., Kapteyn, J., and Simon, J.E. *In vitro* regeneration of *Echinacea purpurea* from leaf explants. Plant Cell Tiss Organ Cult. 69: 79-83, (2002).
21. Kumpulainen, J.T., Salonen, J.T. Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease, The Royal Society of Chemistry,UK, pp. 178-187, (1999).
22. Ludwig-Müller, J. Indole-3-butyric acid in plant growth and development. Plant Growth Regul. 32: 219–230, (2000).
23. Mante, S., Scorza, R. and Cordts, J. M. Plant regeneration form cotyledons of *Prunus persica*, *Prunus domestica* and *Prunus cerasus.* Plant Cell, Tissue Organ Culture 19: 1-11, (1989)
24. Martin, G., Geetha, S.P., Balachandran, I., and Ravindran, P.N. Micropropagation of *Celastrus paniculatus* Willd: A highly sought after medicinal plant in Ayurveda.

Proceedings of the National Symposium on Biotechnology for a Better Future, Jan. 15-17, St. Aloysius College, Mangalore, pp: 43-43, (2004)
25. Mathur, S., Shekhawat, G.S., and Batra, A. Somatic embryogenesis and plantlet regeneration from cotyledon explants of *Salvadora persica* L. Phytomorphology, 58:57-63, (2008)
26. Metivier, P.S.R., Yeung, E.C., Patel, K.R., and Thorpe, T.A. *In vitro* rooting of microshoots of *Cotinus coggygria* Mill, a woody ornamental plant. In Vitro Cell Dev Biol. 43: 119-123, (2007).
27. Murashige, T., and Skoog, F. A revised medium for rapid growth and bioassays with *tobacco* tissue cultures, Physiol. Plant 15.473–497, (1962).
28. Nayak, P., Behera, P.R., and Manikkannan. T. High frequency plantlet regeneration from cotyledonary node cultures of *Aegle marmelos* (L.) Corr. In Vitro Cell Dev Biol. 43: 231-236, (2007).
29. Ozean, S., Burghehi, M., Firek, S., and Draper, J. High frequency adventitious shoot regeneration from Immature colyledons of Pea (*Pisum sativum* L.) Plant cell Rep. 11: 44-47, (1992).
30. Pereira, A.M., and Bertoni, B,W. Appezzato-da-Glória B, Araujo ARB, Januário AH, Lourenco MV, Franca SC. Micropropagation of *Pothomorphe umbellate* via direct organogenesis from leaf explants. Plant Cell Tiss Organ Cult. 60: 47-53, (2000).
31. Powers, S.K., Deruisseau, K.C., Quindry, J., and Hamilton, K.L. Dietary antioxidants and exercise. Journal of Sports Sciences. 22: 81-94, (2004).
32. Pradhan, C., Kar, S., Pattnaik, S., and Chand, P.K. Propagation of Dalbergia sissoo Roxb. through *in vitro* shoot proliferation from cotyledonary nodes. Plant Cell Rep 18:122–126, (1998).
33. Pretto, F.R., and Santarém, E.R. Callus formation and regeneration from *Hypericum perforatum* leaves. Plant Cell Tiss Organ Cult. 62: 107-113, (2000).
34. Raghavaswamy, B.V., Himabindu, K., and Sita, G.L. *In vitro* micropropagation of elite rosewood (*Dalbergia latifolia* Roxb.). Plant Cell Reports, 11: 126-131, (1992)
35. Rajeswari, V., and Paliwal, K. *In vitro* adventitious shoot organogenesis and plant regeneration from seedling explants of *Albizia odoratissima* L.f. (Benth.). In Vitro Cell Dev Biol. 44: 78-83, (2008).
36. Roy, A.T., and De, D.N. Tissue culture and plant immature embryo explants of *Calotropis gigantea* Linn. R.Br. Plant Cell, Tissue Organ Culture 20: 229-233, (1990).
37. Roy, A.T., and De, D.N. *In vitro* plantlet regeneration of the petrocrop *Calotropis gigantea* R.Br. *Proceedings Bioenergy Society Third Convention and Symposium* (Bioenergy Society of India, New Delhi). pp. 123-128, (1986).
38. Salah, N., Miller, N.J., Paganga, G., Tijburg, L., Bolwell, G.P, and Rice-Evans, C. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Archives of Biochemistry and Biophysics, 322 (2): 339 – 346, (1995).
39. Sinha, R.K., Majumdar, K., and Sinha, S. *In vitro* differentiation and plant regeneration of Albizia chinensis (OBS.) MERR. In Vitro Cell Dev Biol Plant 36:370–373, (2000).
40. Sumana, K.R., Kaveriappa, K.M., and Bhat, H.S. *In vitro m*icropropagation of *Holarrhena pubescens*. J Med Arom Plant Sci. 21: 299-303, (1999).
41. Tawfik, A.A., and Mohamed, M.F. Regeneration of salvia (*Salvia officinalis* L.) via induction of meristematic callus. In Vitro Cell Dev Biol. 43: 21-27, (2007).
42. Thomas, T.D., and Puthur, J.T. Thidiazuron induced high frequency shoot organogenesis in callus from *Kigelia pinnata* L. Bot Bull Acad Sini. 45: 307-313, (2004).
43. Tiwari. V., Singh, B.D., and Tiwari, K.N. Shoot regeneration and somatic embryogenesis from different explants of Brahmi [*Bacopa monnieri* (L.) Wettst.]. Plant Cell Rep. 17:538-543, (1998).
44. Xie, D.Y., and Hong, Y. Regeneration of *Acacia mangium* through somatic

embryogenesis. Plant Cell Rep. 20: 34-40, (2001).
45. Yalcin-Mendia, N.Y., Ipek, M., Kacan, H., Curuk, S., Sari, N., Cetiner, S., and Gaba, V. A histological analysis of regeneration in watermelon. J Plant Biochem Biotechnol 12, 147–50, (2003).
46. Zhang, Y., Zhou, J., Wu, T., Cao, J. Shoot regeneration and the relationship between organogenic capacity and endogenous hormonal contents in pumpkin. Plant Cell Tissue Organ Cult 93, 323–31, (2008).
47. Zhang, Z., Hanna, W., and Ozias-Akins, P. Comparison of callus induction and plant regeneration from different explants in triploid and tetraploid turf-type bermudagrasses. Plant Cell Tiss Organ Cult. 90: 71-78, (2007).