In vitro Propagation and Conservation of *Maerua apetala* (Spreng.) M. Jacob

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Abstract

The present investigation focused on *in vitro* propagation of *Maerua apetala* through nodal and shoot tip explants. The explants placed on Murashige and Skoog (MS) medium fortified with either BAP (0.5-3.0mg/l) or BAP (0.5-3.0mg/l) in combination with IAA (0.1-0.6mg/l) and TDZ (0.1-2.5mg/l) produced adventitious shoots. Maximum Shootlets were observed on MS medium supplemented with BAP 1.0 & 1.5 mg/l that produced 80% shoots.The explants inoculated on MS medium with BAP 1.0 mg/l yielded maximum average number of Shoots (14.5±1.18) and mean length of shoots (5.5 cm). The *in vitro* derived micro shoots were subjected to rooting on ½ MS medium with IBA (0.5-2.5mg/l), IAA (0.5-2.5mg/l) and NAA (0.1-2.5mg/l). The Maximum number of roots was observed in IBA 1.0 mg/l that initiated 80% roots and an average of 6.4 ± 0.61^{C} roots.

Keywords: *Maerua apetala*, nodal explants, MS medium, Plant Growth regulator

Introduction

The medicinal tree *Maeraua apetala* belonging to Capparaceae are scattered in Tamil Nadu (Gamble and Fischer, 1915-1936; Hooker, 1872-1897; Matthew, 1981). There are 40 genera and 450 species of herbs, shrubs and rarely trees are distributed nearly equally over tropical and subtropical regions (Caius, 1998). Many of these plants have been used in traditional systems of medicine. *Maerua apetala* has not been explored extensively by the scientific world so far as it has very little previous record of use in traditional medicine.

Maerua apetala is commonly known as '*Iruvali*' in Tamil and is a medium sized tree of scrub jungles and endemic to Tamil Nadu and Andhra Pradesh (Daniel and Uma Maheswari, 2001). Traditionally, the root bark paste is applied for leucoderma and the extract given orally for the same by Chenchus and Lambadies (Reddy *et al.*, 1995). Tender leaves ground with spices and the paste made into pills are given orally for nervous disorders and foot pains by Chenchus (Diallo *et al.*, 2000).

Materials and Methods

The study plant was collected from Gangaikondan Spotted Deer park location. It is a tree about 4 m tall, young branches pale, glabrous; Leaves trifoliate, leaflets elliptic, linear obtuse or rounded at base, coriaceous; Flowers pale green, terminal corymbs, bracteolate; pedicels 2 cm long. Calyx tube 0.3 cm long. Petals absent. Stamens many; gynophores about 1.8 cm long. Ovary ovoid. Fruits ovoid; seeds trabeculate; Flowers and Fruiting season March-July.

The nodal and shoot tip explants were collected from the healthy mother plants, thoroughly washed with running tap water for 10 min. followed by Tween 20 treatments for 10 minutes to remove the superficial dust particles. Then the explants were surface sterilized inside the Laminar Air Flow chamber with 0.1% $HgCl_2$ solution for 3 minutes, followed by rinsing three times with double distilled water. The sterilized nodal segments were implanted vertically on MS medium (1962) fortified with 3% sucrose, 0.6% (w/v) agar and different concentrations and combinations of BAP (0.5-3.0 mg/l), IAA (0.1-0.6 mg/l) and TDZ (0.1-2.5) for shoot induction. Proliferated shoots initiated from nodal segments were sub cultured for further multiple shoot induction. Regenerated multiple shoots were cut and individual shoots were transferred into MS medium containing different concentrations of IBA (0.5-2.5 mg/l), IAA (0.5-2.5 mg/l) and NAA (0.1-2.5 mg/l) for root induction. For the purpose of hardening, the rooted plantlets were thoroughly washed to remove the traces of agar and planted in polycups containing a mixture of soil, sand and farmyard mixture in the ratio of 1:1:1 and covered with perforated plastic bags and hardened for four weeks in a mist chamber before transfer to the field.

Results and Discussion

The nodal and shoot tip explants on MS medium supplemented with BAP (0.5 - 3.0 mg/l), TDZ (0.1 - 2.5 mg/l) and IAA (0.1 - 0.6 mg/l) induced multiple shoots. The MS medium augmented with BAP (1.0 mg/l) induced 80% of multiple shoot formation and maximum number (14.5± 1.18) of shoots per explant was observed. Maximum shoot length of 5.5 cm was observed on the same medium concentration (Table 1 & Plate 1). In the same way Purohit and Kukda (2004) were able to induce multiple shoots from nodal explants of a 30-yr-old tree of *Wrightia tinctoria* on MS medium supplemented with BAP 2.0 mg/l which affirms that BAP alone may also induce multiple shoots. In the same way Isabel *et al*. (2004) formulated a micropropagation protocol for *Helianthemum stropogophyllum*. Shoot tips and nodal segments isolated from seedlings were used as primary explants. Multiple shoot production was observed in MS medium supplemented with different concentrations of BAP. Lee and Chan (2004) also observed multiple shoots from the nodal segments of *Orthosiphon Stamineus* using MS + BAP 0.5 mg/l. However there are reports from Daniela *et al*. (2009) who observed BAP along with NAA yielded maximum number of shootlets from the nodal explants of *Neoglaziovia variegate* on MS media fortified with NAA 0.5 mg/l

and BAP 4.4 mg/l and Anand and Jeyachandran (2004) developed a protocol for *Zehneria scabra* (L.f.) Sonder, through nodal explants cultured on MS fortified with BAP 5 mg/l and IAA 0.5 mg/l and achieved high frequency of multiple shoots induction.

The *in vitro* raised shoots transferred into ½ MS medium supplemented with various concentrations and combinations of IBA, IAA and NAA were kept in darkness for four days. On 5th day the cultures were transferred to normal light condition for rooting. After 10 days, the roots were formed in the shoots on ½ MS medium supplemented with of IBA 1.0 mg/l and it produced maximum number (6.4±0.61) of roots and the rooting response was 80% (Table 2, Plate 1). The increased IBA concentration led to the formation of basal callus. Almost similar results have been observed by Mohapatra *et al.* (2008) who obtained 3-4 roots per shoot by culturing the shoots in MS media with IBA 0.5 mg/l in *Centella asiatica*. Likewise, Chandraprabha and Ramasubbu (2010) established roots in MS media with IBA 1.0 mg/l in *Aristolochia tagala* and Kumar *et al.* (2013) also have developed an *in vitro* propagation protocol for *Citrullus colocynthis* (Linn.) in which rooting was readily achieved upon transferring the shoots on to ½ strength MS medium supplemented with IBA 4.9 mg/l. On the otherhand, there are reports where rooting have been induced by using plant regulators other than IBA or in combination with IBA. Sri *et al. (*2013) have worked on endangered wild medicinal plant *Stemona tuberosa* induced multiple roots containing ½ MS with 1.0 mg/l IAA and Eganathan (2012) have developed a micropropagation protocol for *Sauropus androgynus* (L.) Merr. using nodal explants in MS medium supplemented with various concentrations of BA and Kn and rooting was induced from shoots in MS medium supplemented with various concentrations of IBA and NAA.

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Table-1: Effect of PGRs on induction of multiple shoot from nodal explants of *Maerua apetala*

PGRs			Shooting Response (%)	Average Number of Shoots per explant	Mean shoot length (cm)
BAP	IAA	TDZ			
0.5			75	12.3 ± 1.95^{cd}	4.7^{bc}
1.0			80	14.5 ± 1.18^{a}	5.5^{ab}
1.5			80	14.0 ± 2.36^{ab}	4.3^{cd}
2.0			70	11.2 ± 1.69^{ef}	4.0^{de}
2.5			65	9.3 ± 1.15^{ef}	3.9^{de}
0.5	0.1		70	9.6 ± 2.79^{cd}	4.9^{ab}
1.0	0.2		70	11.5 ± 1.95^{de}	5.2^{ab}
1.5	0.3		70	11.3 ± 1.30^{e}	5.3^{a}
2.0	0.4		65	11.1 ± 1.91^{ef}	5.1^{ab}
2.5	0.5		65	9.8 ± 1.69^{g}	4.3^{cd}
3.0	0.6		60	9.5 ± 1.95^{gh}	3.3^{fg}
		0.1	55	9.8 ± 1.49^{g}	3.0^{ghi}
		0.5	60	11.9 ± 1.52^{de}	2.5^{jkl}
		1.0	65	12.5 ± 1.10^{bcd}	3.1^{fgh}
		1.5	65	12.3 ± 0.96^{cd}	3.9^{de}
		2.0	60	11.4 ± 1.57^{de}	3.6^{ef}
		2.5	60	11.0 ± 1.06^{ef}	2.4^{jkl}

Note: Mean value within the same column followed by the same superscript(s) are not significantly different ($p \leq 0.05$) according to ANOVA and LSD multiple range tests.

Table 2: Effect of PGRs on multiple roots induction from *in vitro* derived shoots of *Maerua apetala* (½ MS medium)

PGRs			Rooting Response (%)	Number of Roots per Shoot	Basal Callus rating
IBA	IAA	NAA			
0.5			65	4.5 ± 1.31^{b}	-
1.0			80	6.4 ± 0.61^{c}	-
1.5			75	5.5 ± 0.4^{ab}	-
2.0			48	3.0 ± 1.31^{ab}	-
2.5			-	0.0^{a}	++++
	0.5		-	0.0^{a}	-
	1.0		46	2.4 ± 0.50b	-
	1.5		62	4.5 ± 1.61^{c}	-
	2.0		42	3.6 ± 0.56^{b}	-
	2.5		-	0.0^{a}	+++
		0.1	-	0.0^{a}	-
		0.5	55	1.2 ± 0.34^{c}	-
		1.0	60	3.0 ± 0.74^{c}	-
		1.5	65	4.8 ± 0.58^{c}	-
		2.0	60	2.0 ± 0.46^{c}	-
		2.5	-	0.0^{a}	++++

Note: Mean value within the same column followed by the same superscript(s) are not significantly different ($p \leq 0.05$) according to ANOVA and LSD multiple range tests.

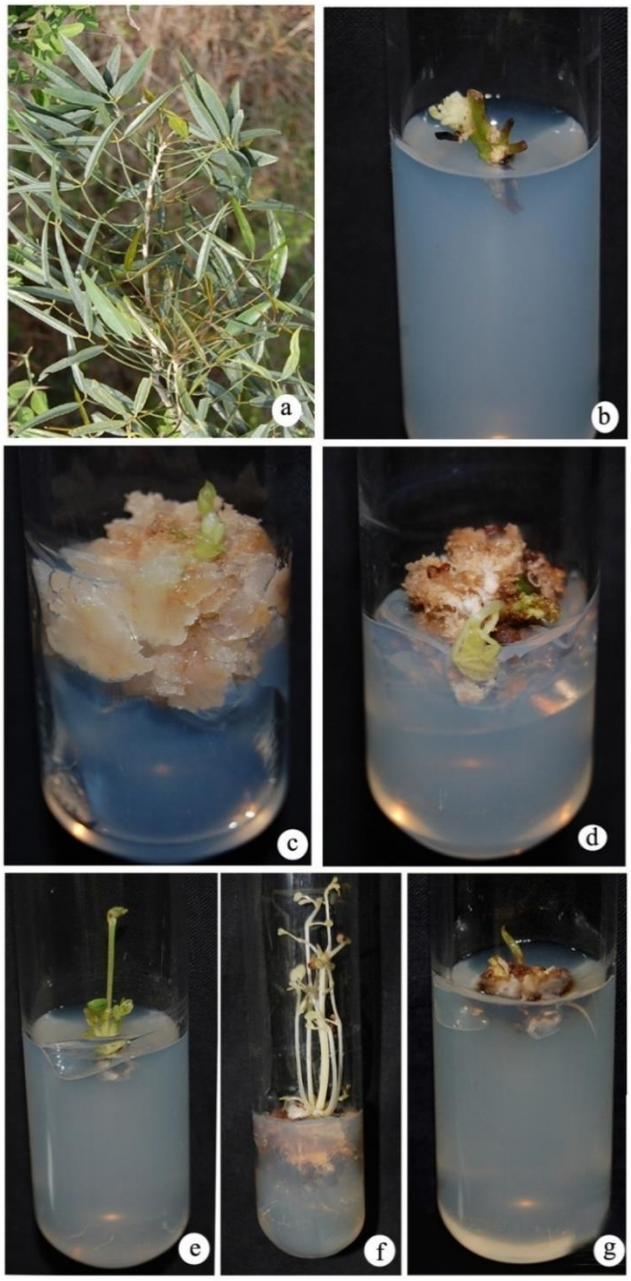
References

1. Anand, S. D. and Jayachandran, R. (2004). *In vitro* multiple shoot regeneration from nodal explants of *Zehneria Scabra* (LF) Sonder. *Plant Tissue Culture*, 14: 101-106.
2. Caius, J. F. (1998). *Cadaba trfoliata* In: *The Medicinal and Poisonous Plants of India*. Scientific Publishers (India), Jodhpur, pp. 267-269.
3. Chandraprabha, A. and Rama Subbu, R. (2010). Micropropagation of *Aristolochia tagala* Cham. - a Rare and Endemic Medicinal Plant from Western Ghats, Standard Fireworks Rajarathnam College for Women, Sivakasi, India Vol. 1: 70-73.
4. Daniel, P. and Uma Maheswari, P. (2001). *Maeura*. In: *The Flora of the Gulf of Mannar*. BSI, Kolkatta: pp. 62-64.
5. Daniela Garcia Silveira, Fernanda Vidigal Duarte Souza, Claudinéia Regina Pelacani, Antonio da Silva Souza, Carlos Alberto da Silva Ledo and José Raniere Ferreira de Santana (2009). Micropropagation and *in vitro* conservation of *Neoglaziovia variegata* (Arr. Cam.) Mez, a fiber producing bromeliad from Brazil. *Braz. Arch. Biol. Technol*. 52: 46-52.
6. Diallo, D., Sogn, C., Samake, F. B., Paulsen, B.S., Michaelsen, T. E, Keita, A. (2000). Wound Healing Plants in Mali the Bamako Region: An Ethanobotanical Survey and Complement Fixation of Water Extract from Selected Plants. *Pharmaceutical Biology*, 40: 117-128.
7. Gamble, J.S. and Fischer, C.E.C. (1915-36). *Flora of the Presidency of Madras*. London Vol I,II & III.
8. Hooker, J. D. (ed.) (1872-97). *The Flora of British India* (7th Vol.), London.
9. Isabel Santana Lopez, J. C., Luis Margarita Ravel Arms and Francislo valises Gonzalez (2004). *In vitro* propagation of *Heliantheme bystropogophyllum* (Cistaceae) Svent.: A rare and endangered species from gram canaries (Canny islands). *Bot. Macaronesica*, 25: 71-77.
10. Kumar Sambhav Verma, Sumita Kachhwaha and Kothari, S. L. (2013). *In vitro* plant regeneration of *Citrullus colocynthis* (L.) Schard. and Assessment of Genetic Fidelity using ISSR and RAPD Markers. *Ind. J. Biotech*,, 12: 409-414.
11. Lee, W. L. and Chan, L. K. (2004). Establishment of *Orthosiphon stamineus* cell suspension culture for cell growth. *Plant Cell, Tissue and Organ Culture*, 78: 101-106.
12. Matthew, K. M. (1981). *Materials for the Flora of Tamil Nadu Carnatic*. Tiruchirappalli, India.
13. Mohapatra, H., Barik, D. P. and Rath, S. P. (2008). *In vitro* Regeneration of Medicinal Plant *Centella asiatica*. *Biologia Plantarum.* 52: 339-342.
14. Purohit, S. D. and Kukda, G. (2004). Micropropagation of adult tree *Wrightia tinctoria* R. Br. *Indian J. Biotech.*, 3: 216-220.

15. Reddy, R. V. (1995). *Ethnobotanical and Phytochemical Studies on Medicinal Plant Resources of Cuddapah District, A.P., India*. Ph.D. Thesis, S.K, University, Anantapur, India.
16. Sri Rama Murthy, K., Chandrasekhara Reddy, M., Kondamudi, R. and Pullaiah, T. (2013). Micropropagation of *Stemona tuberosa* Lour.: An Endangered and Rare Medicinal Plant in Eastern Ghats of India. *Ind. J. Biotech.*, 12: 420-424.

Plate - 1

Micropropagation of *Maerua apetala* (Roth) Jacobs



a) Habit ; b) Shoot initiation from nodal eplant ;
c - d) Basal callus formation from nodal explant ;
e - f) Multiple shoots induction & elongation ; g) Root initiation