Academic Journal of Plant Sciences 3 (1): 19-22, 2010
ISSN 1995-8986

Quick *in vitro* Plant Regeneration from Immature Seeds of *Murraya koenigii* (L.) Spreng

Uzma Rani, M.M. Sharma, Najma Ismail and Amla Batra

Department of Botany, Plant Biotechnology Lab. No. 5, University of Rajasthan, Jaipur, India

Abstract: An efficient and reproducible *in vitro* protocol for the large scale propagation of *Murraya koenigii* (L.) Spreng. from immature seeds has been described. Direct shoot proliferation was achieved from intact seedlings on Murashige and Skoog (MS) medium supplemented with BAP (2.5 mg l^{-1}). The multiple shoots (19.85±0.10) raised were dwarf and they were transferred to shoot elongation medium comprising of MS salts fortified with GA_3 (0.4 mg l^{-1}). The elongated shoots (5.6±0.35 cm) were then rooted on half-strength MS medium supplemented with IAA (0.5 mg l^{-1}). The complete plantlets were hardened and acclimatized in plant growth chamber at 26±2°C temperature, 80% humidity and control light about 3000 lux. Finally, the plantlets were transferred in the field conditions, where they were survived with 80% survival rate.

Abbreviations: BAP - 6-benzyladeninopurine, IAA– indole-3-acetic acid, IBA –Indole -3- butyric acid, Kn- 6- furfurylaminopurine, GA_3-Gibberllic acid, MS – Murashige and Skoog's medium

Key words: *In vitro* propagation · Immature seeds · *Murraya koenigii* (L.) Spreng

INTRODUCTION

Murraya koenigii (L.) Spreng. commonly known as curry leaf tree belongs to family Rutaceae. The plant oil contains about 39 compounds of which the major are 3-carene (54.2%) and caryophyllene (9.5%), They are being used to control diabetes, leprosy, gastrointestinal diseases, vomiting, diarrhoea, skin diseases and dysentery and they also act as a fixative for a heavy type of soap perfume [1-5]. The leaves are mainly employed as flavouring agent in curries due to the presence of aroma components like non terpenoids acyclic â ketones and are also the important source of vitamin A and C. Its aqueous extract is hyperglycemic [6]. The root extract also attributes many medicinal properties like anti-bacterial, anti-inflammatory and anti-feedant etc. which have been used to relieve pain associated with kidney [7].

The conventional method of propagation of this tree is limited to seeds, which germinate under partial shade conditions. The seeds retain their viability only for a short period (one month) and whenever the seeds germinate, the young seedlings lead to death under natural conditions [8].

A very sparse work regarding the micropropagation of this important aromatic and medicinal plant has been done by using intact seedlings [9] and nodal segments [10]. However, there are several limitations observed during the plant regeneration by adopting the earlier established protocols. The present paper describes a simple, reliable and repeatable protocol for the *in vitro* regeneration of *M. koenigii* L. spreng by using immature seeds.

MATERIALS AND METHODS

Explant Source: Immature fruits of *Murraya koenigii* (L.) Spreng were collected in the month of July, from the Botanical garden, University of Rajasthan, Jaipur. Seeds from immature fruits were carefully taken out by removing the pulp of fruits with the help of scalpel and then washed in running tap water for about 5 minutes and then kept in teepol 1% (v/v) and 0.02% (w/v) solution of Bavistin fungiside for about 2 minutes followed by rinsing with sterilized double distilled water for 5-6 times so as to remove all traces of sterillant. They were then surface sterilized with 2% sodium hypochlorite (NaOCl; v/v) and 0.1% mercuric chloride ($HgCl_2$, w/v) for 2 minutes respectively and then rinsed with double distilled water at least thrice.

Culture Media and Conditions: Murashige and Skoog medium (1962) [11] containing 3% sucrose and 0.8% agar along with various growth regulators like auxins e.g. IAA, IBA (0.1-2.0 mg l^{-1}) and cytokinin such as BAP, Kintein

Corresponding Author: Uzma Rani, Department of Botany, Plant Biotechnology lab. no. 5, University of Rajasthan, Jaipur, India

(0.5 to 4.0 mg l^{-1}) was prepared. The pH of the medium was adjusted to 5.8±0.2 prior to autoclaving at 121° C for 15 minutes. For each experiment, 8 replicates were prepared and each experiment was repeated at least thrice.

After the preparation and solidification of media, the sterilized explants were then inoculated on the nutrient medium and the cultures incubated at 25±2°C, humidity (50±5%), 16/8 hours photoperiod (3000 lux) provided by white, cool, florescent tubes and dark period respectively.

Shoot Proliferation, Multiplication and Elongation: For *in vitro* shoot induction from immature seeds, various concentrations (0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 mg l^{-1}) of different cytokinins (BAP, Kn) alone were tested. BAP at a concentration of 2.5 mg l^{-1} produced 19.85±0.10 shoots per explant. The shoots regenerated on the BAP were dwarf. Moreover, these were then subcultured for their elongation on MS medium supplemented with GA_3 (0.1-0.6 mg l^{-1}) along with BAP (2.5 mg l^{-1}).

Rooting, Hardening and Acclimatization: The *in vitro* elongated shoots then aseptically transferred on half strength MS medium supplemented with various concentrations (0.1-2.0 mg l^{-1}) of different auxins (IAA, IBA) alone. For *in vitro* root induction and proliferation, IAA (0.5 mg l^{-1}) proved to be the best for rooting. The plantlets were then gently picked from culture vessel without damaging the delicate root system and then rinsed with distilled water to remove adhering agar. The plantlets were transferred to earthen pots containing vermicompost and autoclaved soil (1:3). Plants were covered with inverted glass beakers to maintain high humidity and kept in culture chamber, where all the physical conditions like, humidity, temperature and light was provided artificially. Plantlets were gradually exposed to natural conditions for their acclimatization, each day a few hours.

RESULTS AND DISCUSSION

During the present research investigation, multiple shoots induced when immature seeds inoculated on MS medium supplemented with BAP (2.5 mg l^{-1}), further increased concentration of BAP beyond 2.5 mg l^{-1} decreased the number of shoots (Plate-1, Fig. A; Table 1). Initially 10-12 shoots were proliferated after two subculturing, the number of multiple shoots increased upto (19.85±0.10) after four subculturing (Plate-1, Fig. B). There has been 15 days interval between every subculture. Of the two cytokinins used, BAP induced maximum number of multiple shoots as compared to Kn.

Table 1: Effect of cytokinins on shoot proliferation via immature seeds in *Murraya koenigii* (L.) Spreng after four weeks of subculturing.

Growth regulators (mg l^{-1})	% of shoot formation	No. of shoot bud per explant Mean ±S.D.
BA		
0.0	0	
0.5	35	0.84±0.13
1.0	46	1.28±0.05
1.5	55	2.30±0.37
2.0	64	7.47±0.21
2.5	90	19.85±0.10
3.0	73	11.47±0.18
4.0	60	7.38±0.08
Kn		
0.0	0	
0.5	20	1.00±0.42
1.0	33	2.12±0.16
1.5	54	4.25±0.13
2.0	48	3.22± 0.15
2.5	36	2.28±0.13
3.0	27	1.10±0.35
4.0	15	0.90±0.15

Values represent mean±SE of 28 replicates per treatment in three repeated experiments. Mean values within the column followed by the same letter are not significantly different by the Tukey's test at 0.05% probability level

Table 2: Effect of Gibbrellic acid on elongation of *in vitro* proliferated shoots

Growth regulators (mg l^{-1})	Shoot length (cm.)
BA + GA_3	
2.5+ 0.1	3.5±0.16
2.5+ 0.2	3.4±0.42
2.5 + 0.3	4.2±0.32
2.5+ 0.4	5.6±0.35
2.5+ 0.6	4.5±0.08

Values represent mean±SE of 28 replicates per treatment in three repeated experiments. Mean values within the column followed by the same letter are not significantly different by the Tukey's test at 0.05% probability level

Table 3: Effect of auxins on root formation from the *in vitro* cultured on ½ MS medium

Growth regulators (auxin) (Mg l^{-1})	% of rooting	Root length (cm.)
IAA		
0.1	65	2.30±0.16
0.5	78	3.36±0.21
1.0	50	2.03±0.24
1.5-2.0	Nil	
IBA		
0.1	25	0.30±0.16
0.5	35	1.88±0.09
1.0- 2.0	Nil	

Values represent mean±SE of 28 replicates per treatment in three repeated experiments. Mean values within the column followed by the same letter are not significantly different by the Tukey's test at 0.05% probability level

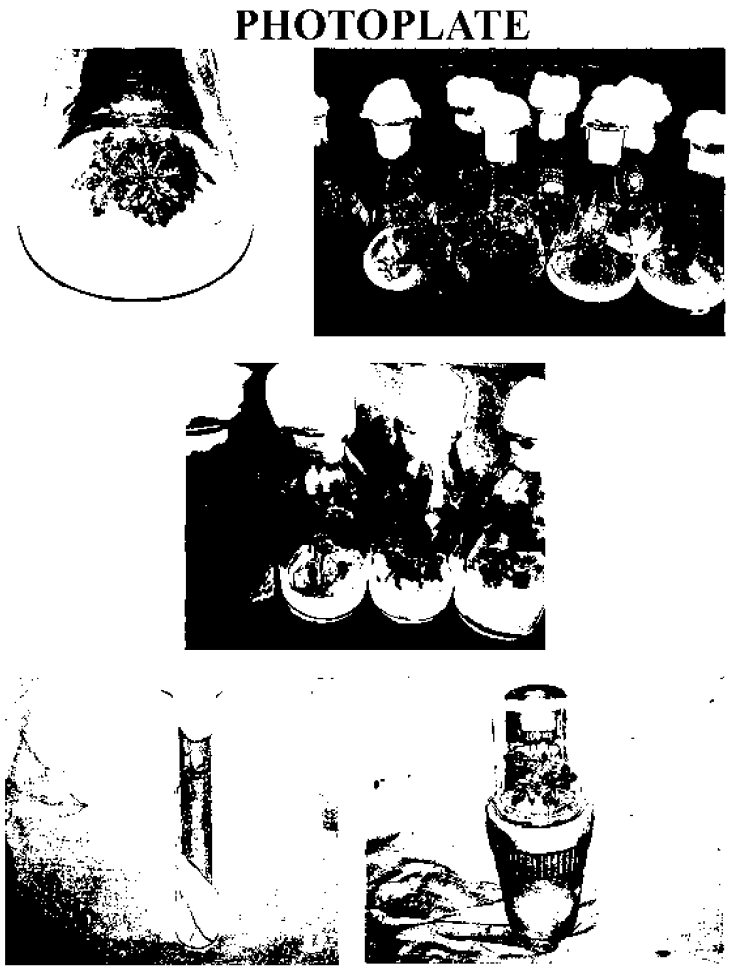

Fig. 1A-E : A: Multiple shoot proliferation from the immature seeds on MS medium along with BAP (2.5 mgl^{-1})
B: Multiplication shoot on MS medium along with BAP (2.5 mgl^{-1}) after four subculturing
C: Elongation of shoots on MS medium supplemented with BAP (2.5 mgl^{-1}) and GA_3 (0.4 mgl^{-1})
D: Rooting on *in vitro* raised shoots on half strength of MS medium supplemented with IAA (0.5 mgl^{-1})
E: Hardening of plantlet in earthen pot after 3 weeks

Similar observations were reported in several other plants such as *Feronia limonia* L. Swingle Hiregoudar *et al*., [12]; *Aegle marmelos* L. Das *et al.* [13]. In contrast, Nayak *et al*., [14] observed that BAP along with IAA was optimum for *in vitro* shooting in *Aegle marmelos* L.

Multiple shoots raised on MS medium augmented with BAP (2.5 mg l^{-1}) were dwarf and they were elongated on the MS medium supplemented with optimized BAP along with GA_3 (0.4 mg l^{-1}) (Plate-1, Fig. C; Table 2). In consonance to the present study, Hussain *et al*., [15] reported elongation of shoots on GA_3 in *Sterculia urens*. However in plant like *Ruta graveolens L*., Bohidar *et al*., [16] reported the elongation of shoots on the same medium. In contrast to the above, elongation has been completed on the medium having kinetin in place of GA_3 in *Pueraria tuberosa* by Rathore and Shekhawat [17]. About 5.5-6.0 cm elongated shoots transferred to root induction medium comprising of MS salts along with IAA (0.5 mg l^{-1}), which induced *in vitro* rooting (3.36±0.21) at the basal end of the shoots after 15 days of culture (Plate-1, Fig. D). The roots were thick, white in colour and having tiny root hairs. Further, IBA also induced rooting but the roots were not healthy for absorbing the nutrients (Table 3).

Earlier Mustafa *et al*., [18] in *Phellodendron amurense* supported the present results on *in vitro* rooting. In oppugance to the rooting on IAA, IBA and NAA also proved to be a optimum root inducing hormone in *Aegle marmelos* L. by Das *et al.*, [13] and *Citrus reticulate* by Mukhtar *et al*., [19] respectively.

After the complete plantlet regeneration, these were hardened and acclimatized by the procedure mentioned in "materials and methods (Plate-1, Fig. E)."

Hence, an efficient and reproducible protocol was developed for *in vitro* regeneration of *Murraya koenigii* (L.) Spreng from immature seeds which would be helpful to provide continuous supply of plants to fulfill the needs of various pharmaceutical industries.

ACKNOWLEDGEMENT

Special thanks to Mr. Roop Narayan Verma for the preparation of photo plates.

REFERENCES

1. Chowdhury, J.U., M.N.I. Bhuiyan and M. Yusuf, 2008. Chemical composition of the leaf essential oils of *Murraya koenigii* (L.) Spreng and *Murraya paniculata* (L.) Jack. Bangladesh Journal Pharmacology, 3: 59-63.
2. Yadav, S., V. Vats, Y. Dhunnoo and J.K. Grover, 2002. Hypoglycemic and anti-hyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. Journal of Ethnopharmacology, 82: 111-116.
3. Manandhar, N.P., 2002. Plant and People of Nepal. Timber Press Inc. Portland, Oregon.
4. Baral, S.R. and P.P. Kurmi, 2006. A compendium of medicinal plants in Nepal. Mrs. Rachna Sharma Publication, Kathamandu.
5. Joshi, S.G., 2006. Medicinal Plants. Oxford and IBH Publishing, New Delhi.
6. Kesari, A.N., R.K. Gupta and G. Watal, 2005. Hypoglycemic effects of *Murraya koenigii* on normal and alloxan diabetic rabbits. Journal of Ethnopharmacology, 97: 247-251.
7. Anonymous, 1988. Wealth of India. A dictionary of Indian raw materials and industrial products, Vol. VI (L-M). CSIR Publication, New Delhi, India, pp: 446-448.
8. Rout, R.G., 2005. Direct Plant Regeneration Of Curry Leaf Tree (*Murraya Koenigii* Koenig.), An Aromatic Plant. *In Vitro* Cellular and Developmental Biology-Plant, 41(2): 133-136.
9. Bhuyan, A.K., S. Pattnaik and P.K. Chand, 1997. Micropropagation of Curry Leaf Tree [*Murraya koenigii* (L.) Spreng.] by axillary proliferation using intact seedlings. Plant Cell Report, 16: 779-78.
10. Babu, N.K., A. Anu, A.B. Remashree and K. Praveen, 2000. Micropropagation of curry leaf tree. *Plant Cell* Tissue and Organ Culture, 61: 199-203.
11. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plantarum, 15: 473-479.
12. Hiregoudar, L.V., B.P. Murthy, E.J. Hema, Hahn and K.Y. Paek, 2003. Multiple shoot induction and plant regeneration of *Feronia limonia* (L.) Swingle. Scientia Horticulturae, 98(4): 357-364.
13. Das, R., M.F. Hasan, M.S. Rahman, M.H. Rashid and M. Rahman, 2008. Study *on in vitro* propagation through multiple shoot proliferation in wood apple (*aegle marmelos* l.). International Journal Sustainaible Crop Production, 3(6): 16-20.
14. Nayak, P., P.R. Behera and T. Manikkannan, 2007. *High frequency plantlet regeneration from cotyledonary node cultures of Aegle marmelos (L.) Corr. In Vitro* Cellular and Developmental Biology-Plant, 43(3): 231-236.
15. Hussain, T.M., T. Chandrasekhar and G.R. Gopal, 2008. Micropropagation of *Sterculia urens* Roxb., an endangered tree species from intact seedlings. African Journal of Biotechnology, 7(2): 95-101.
16. Bohidar, S., M. Thirunavoukkarasu and T.V. Rao, 2008. Effect of plant growth regulators on *in vitro* micropropagation of 'Garden Rue' *(Ruta graveolens L.).* International Journal of Integrative Biology, 3(1): 36-43.
17. Rathore, M.S. and N.S. Shekhawat, 2009. Micropropagation of *Pueraria tuberosa* (Roxb. Ex Willd.) and determination of puerarin content in different tissues. Plant Cell Tissue and Organ Culture, 99(3): 327-334.
18. Mustafa, A.K.A., S. Yokota, T. Ohkubo, Y. Andoh, S. Yahara and N. Yoshizawa, 2004. Micropropagation of Plantlets through Callus in Kihada (*Phellodendron amurense* Rupr.). Shokubutsu Kojo Gakkaishi, 16: 122-130.
19. Mukhtar, R., M.M. Khan, B. Fatima, M. Abbas and A. Shahid, 2005. *In Vitro* Regeneration and Multiple Shoots Induction in *Citrus reticulata* (Blanco). International Journal of agriculture Biology, 7(3): 414-416.