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HIGH FREQUENCY SHOOTS REGENERATION FROM *IN VITRO* RAISED SEEDLINGS OF *MURRAYA KOENIGII* (L.) SPRENG.

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ABSTRACT

Immature seeds of *Murraya koenigii* (L.) were allowed to grow in *in vitro* condition on ½ strength Murashige and Skoog (MS) medium. Cotyledonary node of 3-4 weeks old seedlings were taken and further cultured on Murashige and Skoog (MS) medium supplemented with BAP (5.0 mg/l) for multiple shoots with (15.85±1.0) proliferation. Shoots were separated carefully and sub-cultured to induce roots on ½ strength MS medium supplemented with IBA (1.5mg/l). Regenerated plants were successfully hardened and acclimatized with 80 % survival rate under natural conditions after transplantation.

KEY WORDS

Murraya koenigii, multiple shoots, nodal explant.

ABBREVIATIONS

BAP – 6-Benzyladenino Purine, IBA- indole-3-butryic acid, 2,4-D- 2,4-dichlorophenoxyacetic acid, IAA-indole-3-acetic acid, MS medium – Murashige and Skoog medium.

INTRODUCTION

Murraya koenigii (L.) Spreng. (Family-Rutaceace), commonly known as curry leaf tree is used as a spice throughout India. It is basically known for its immense aromatic value. The traditional medicinal literature describes its potential role as a source of essential vitamins and are used as a domestic remedy for many diseases like diabetes, cancer etc. The leaves, bark and the roots are used intensively in indigenous medicinal system from ancient times, as a tonic for stomachache, stimulant and carminative (Pruthi, 1998). The *Murraya koenigii* leaves are used in traditional medicine for the treatment of piles, headache, stomachache, influenza, rheumatism, traumatic injury, insect and snake bites, antivomitting, curing dysentery and diarrhoea (Chakraborty *et al*., 1965; Kong *et al.,* 1986). It has been reported that carbazole alkaloids present in the plant possesses various biological activities such as anti-tumour, antioxidative, anti-mutagenic and anti- inflammatory etc., respectively (Tachibana *et al., 2001;* Ramsewak *et al*., 1999; Nakahara *et al*., 2002).Conventionally, this particular plant grows via the seed, but they germinate in partial shade. The viability of seeds is limited only to a few weeks, the seedling emerges from the germination of seeds, leads to death in natural environment.

Few reports are available on the regeneration of this particular plant species through the culture of nodal segments (Babu *et al*., 2000), and intact seedling (Bhuyan *et al*., 1997). Here we have standardize tried to a protocol for regeneration of this potent aromatic and medicinal plant through the culture of cotyledonary node.

MATERIALS AND METHODS

Explant: Fruits of *Murraya koenigii* (Rutaceae) were obtained in the month of July from the Botanical garden, University of Rajasthan, Jaipur. Seeds were first washed in running tap water for 2 minutes and then disinfected with teepol 1%(v/v) and 0.02%(w/v) solution of bavistin fungicide 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 hypochloride (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. These sterilized seeds were then aspectically inoculated on 1/2 strength MS Meduim. Cotyledonary nodes were isolated from the 3-4 weeks old seedlings were taken and were used further as a explants source.

Medium was prepared using the MS salt composition, sucrose (3%) and bacteriological grade agar (0.8%), growth regulators were incorporate into the medium and the pH was adjusted to 5.8±1. The medium was then sterilized in an autoclave at 15lb pressure and 121ºC for 20 minutes. The culture conditions maintained for *in vitro* culture were 25±2 ºC, 55-60% relative humidity and 16 hours photoperiod with fluorescent light followed by an 8 hours dark period.

For each experiment of replicated were taken. Experiments were repeated thrice.

***Initiation and proliferation of cotyledonary node*:** Cotyledonary node explants were cultured on MS medium alone or supplemented with 6-benzylamino purine (0.5 - 7.0 mg/l), kinetin (0.5 - 7.0 mg/l) individually. BAP at 5.0 mg/l produced maximum responses. Response, viability, intensity and quantity of shoots development and quality of shoots were recorded weekly.

***Rooting, Hardening and Acclimatization*:** The *in vitro* developed and elongated shoots then aseptically transferred on half strength MS medium supplemented with various concentrations (0.1-2.5 mg/l) of different auxins (IBA, IAA) alone for *in vitro* root induction, IBA (1.5mg/l) proved to be the best for rooting. The plantlets were then gently picked from cultures vessels without damaging the delicate root system and then rinsed with distilled water to remove adhering agar. The plantlets were transferred to vermicompost and sterilized soil (1:3). Plants were covered with inverted glass beakers to maintain high humidity and kept in culture chamber, as well as mist chamber was also used where all the physical condition like, humidity (38%), temperature (26.7 ºC) and light was provided artificially. Plantlets were gradually exposed to natural condition for their acclimatization.

RESULT AND DISCUSSION

Murraya koenigii (L.) spreng. is an aromatic plant with immense medicinal value. Germination of immature seeds were allowed to grow in *in vitro* conditions on 1/2 strength MS medium. Similar results were observed in *C.ternatea* by Shahzad *et al*., (2007). However, in *Psoralea corylifolia* seed germination was observed in MS medium by Jeyakumar and Jayabalan(2002).After 3-4 weeks, Cotyledonary node was taken as a source of explant from developed seedlings and cultured on MS medium with various concentrations of cytokinin (BAP, Kn). However, BAP at 5 mg/l produced maximum number of shoots (**15.85±1.0**) (Graph, 1).Similarly, developed multiple shoots were elongated on the same medium by Mallikarjuna and Rajendrudu (2009) with, on 4 mg/l BAP. Nevertheless, Jeyakumar and Jayabalan (2002) observed the multiplication of shoot on 1mg/l BAP in plant *Psoralea*

*corylifolia,*respectively.The developed and elongated shoots were then transferred on the rooting medium. Among the auxins tried IBA (1.5mg/l) produced best and elongated roots (**3.34 ± 0.04) (Table 2)** . Similar results were also observed *Drymaria cordata* by Ghimere *et al.,(*2010) respectively, where also IBA was best for *in vitro* rooting. However IAA was proved to be optimum for rooting in plants like *Cardiospermum halicabum* (Jahan and Anis 2009;) and *Azadiractha indica* (Shekhawat *et al*., 2006) respectively. Moreover roots were also induced at the cut ends of shoots on MS medium devoid of growth regulators by Tyagi *et al*.,(2010).

Figure

Fig. A- Germination of immature seeds on 1/2 MS medium.
Fig. B- Multiple shoot regeneration from cotyledonary node of M. koenghii on MS + BAP (5.0 mg/l).
Fig. C- Advanced stage of culture showing exposed view of shoot multiplication on BAP (5.0 mg/l).
Fig. D- Shoot elongation and multiplication after 4th subculture.
Fig. E- In vitro rooting on 1/2 MS + IBA (1.5 mg/l).
Fig. F- Hardened plant in mistchamber.
Fig. G- Acclimatized plant in an earthen pot.

Table 1

Effect of growth regulators on shoot proliferation and number of shoots per culture from cotyledonary nodal explants. Data (Mean ± S.D.) were recorded after four weeks.

Growth regulators (mg/l)	% of shoot formation	Mean number of shoots produced/ explants (*Mean ± t 0.05 S.E.)
BA		
0.0	-	-
1.5	50	4.84 ± 0.13
2.0	65	5.38 ± 0.08
2.5	72	6.30 ± 0.37
3.0	74	6.47 ± 0.18
3.5	79	8.75 ± 0.10
4.0	86	7.28 ± 0.05
4.5	89	8.50 ± 0.3
5.0	**90**	**15.85±1.0**
5.5	60	2.60± 0.16
6.0	50	2.57± 0.10
Kn		
0.0	-	-
1.5	45	2.89 ± 0.12
2.0	50	2.10 ± 0.35
2.5	62	4.80 ± 0.42
3.0	65	5.12 ± 0.16
3.5	69	5.58 ± 0.13
4.0	81	6.90 ± 0.15
4.5	62	5.05±0. 20
5.0	62	4.80±0.10
5.5	55	3.75±0.01
6.0	50	2.40±0.11

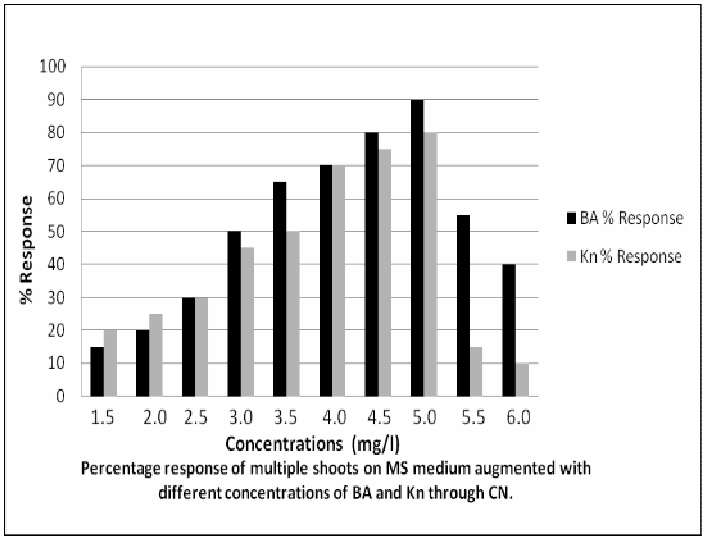
Table 2

Effect of different concentration and combination of auxins on adventitious root formation from the in vitro grown cultured on ½ MS medium. Data (Mean ± S.D.) were recorded after four weeks.

Growth regulators (types auxin)(mg/l)	% rooting response	Av. Length of root (*Mean ± t 0.05 S.E.)
IBA		
0.0	-	-
0.1	68	0.49 ± 0.13
0.5	55	1.79 ± 0.14
1.0	72	2.30 ± 0.03
1.5	**80**	**3.34 ± 0.04**
2.0	73	2.49 ± 0.13
2.5	65	0.97 ± 0.14
IAA		
0.0	-	-
0.1	55	0.96 ± 0.04
0.5	65	1.98 ± 0.07
1.0	70	2.79 ± 0.17 -
1.5	62	1.59 ± 0.12
2.0	63	0.81 ± 0.15
2.5	53	0.09 ± 0.14

Graph 1

Effect of growth regulators on shoot proliferation and number of shoots per culture from cotyledonary nodal explants. Data (Mean ± S.D.) were recorded after four weeks.



In vitro raised plantlets were taken out from the culture vials and hardening and acclimatization was done by the method described in "Materials and Methods". The success of this protocol offers a highly efficient method for mass clonal propagation of *Murraya koenigi, w*hich can be a sustainable system *in vitro* transferred to *in vivo* condition for pharmaceutical industries.Physical and environmental conditions play a vital role in the growth and development of any plant species *in vitro*. However, due to the present studies the regeneration frequency can be affected by photoperiod. We found that explants were regenerative under the 16 hours photoperiod (70.6±1.0%) as compared to those under continous light (50.6±1.0). However regeneration was inhibited regardless of 16 hours photoperiod with high concentration of BAP. Similarly the *in vitro* regeneration frequency affected by light condition and growth regulators, as demonstrated by (Seibert and Kadkade, 1980 Murashige, 1994; Rout 2005). earlier.

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