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CALLUS INDUCTION IN A MEDICINAL PLANT *MURRAYA KOENIGII* SPRENG

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ABSTRACT
Callus proliferation occured on leaf and stem. Explant cultured on Murshige and Skoog's medium (MS) supplemented with 2, 4-D, IBA, NAA and BAP separately or in combination. In the beginning green callus was observed which turned into light yellow and was followed by formation of brown callus. Sometime light green callus also observed. Development of callus was observed from different concentration of auxin or cytokinin alone or in combination callus from shoot tips and nodal was that 2, 4-D (2.5mgL^{-1}) and IBA (0.5mgL^{-1})

INTRODUCTION

Murraya Koenigii Spreng is an aromatic shrub tree found throughout tropical and subtropical East Asia from India and Australia (Xie *et al.*, 2006). In India it is found in the Andaman Island up to altitude of 1,500m recorded its wild form in the Garwhal Hills in Uttar Prades, Sikkim, Bengal and Kerala. *Murraya Koenigii* Spreng (Rutaceae) commonly known as Mitha Neem, curry leaf an important medicinal plant. The leaves are pinnate, leaflets mostly ovate, crenate-dentate, flowers while in crymbose cymes, berries purplish black (Jayaweera, 1982). The major aroma constituents in the oil as b-caryophyllene, b-gurjunene, b-elemene and b-phellandrene. The leaves are extensively used for flavouring curries, soups pickles and condiment. It is also used in many of the Indian ayurvedic and Unani prescription. Curry leaves have high potential as reduces the toxicity of dimethys hydrazine hydrochloride. so, it is called anticarcinogenic. It is also used against diabetes. Curry leaf increased high density lipoproteins and lowered the release of lipoprotein into the circulation (Khan *et al.*, 1996 and 1998) *Murraya Koenigii* Spreng. is a seed grown annual plant and is not propogated by cutting. Tissue culture and micro-propogation protocols have been described for a number of woody species (Roussos *et al.*,1999). This paper describes the development of green, light green and brown callus shows induction of shoot differentiation.

MATERIALS AND METHODS

Green young leaves explants of 4 - 8 months old *Murraya Koenigii Spreng.* were collected from Namkom Plandu and our garden Ranchi. They were washed with running tap water and 1 - 2 drops of Savlon for 2 minutes. They were surface sterilized in 70% ethanol for 30 seconds and immersed in 0.1% $HgCl_2$ for ½ minutes. Then rinsed with autoclaved distilled water (5 times) Leaves and Nodal parts were inoculated in test tube containing MS basal media Murashige Skoog (1962). Callus having different morphology was also used as culture material.

Culture medium

Solid MS medium containing 3% sucrose with varying concentration of 2, 4-D,. NAA, IBA was used for callus formation and shoot regeneration. Combination of auxin (IBA) and Cytokinin (BAP) was also used for plant regeneration. Coconut water Cw - 20% V/V was also used in MS basal medium for regeneration system. The pH of the media was adjusted to 5.8 before gelling with agar (0.8% w/v) and autoclaved for 15 to 20 minutes at 15^{PSI} at 120ºC. The leaf stem and nodal portion were inoculated into incubated in culture room.

Culture condition

Cultures were incubated at 25 $\pm$ 2ºC under cool fluorescent light (1500 – 2000Lux) with a 16 h/8 h light dark cycle. Each treatment consisted of minimum 20 explants and all experiments were repeated at least 7 times. Shoot tip, nodal parts and young leaf plants were cultured on MS medium supplemented with 2, 4-D (0.5 - 5.0mgL^{-1}), NAA (0.5 - 5.0mgL^{-1}), BAP (0.5 - 5.0mgL^{-1}) and IBA (2.5 mgL^{-1}) + BAP (2.5mgL^{-1}) for callusing and for regeneration.

RESULTS AND DISCUSSION

Generally explants taken from young plants of 4 - 8 months,

Table 1: Effects of various concentrations of auxin and coconut water 20% V/V supplemented in MS Medium on callus induction from leaf of *Murraya Koenigii* Spreng. after 5 weeks of culture

Phytohormones added to MS Concentration in MgL^{-1} (MS + 2,4-D + Diff. conc. of Auxin (IBA) + CW 20% v/v	% culture showing callus formation 1st Week	2nd Week	3rd Week	4th Week	5th Week
0.5	8	7	8	8	8
1.0	17	16	17	17	17
1.5	36	34	35	36	36
2.0	76	76	77	76	77
2.5	98	96	99	98	98
3.0	83	81	80	81	81
3.5	62	63	62	63	63
4.0	54	55	56	54	55
4.5	53	52	53	52	53
5.0	45	47	46	45	46

Table 2: Effects of various concentrations of cytokinin and combination of cytokinin and auxin on callus induction and shoot differentiation from leaf and nodal culture of *Murraya Koenigii* Spreng. after 5 weeks of culture

MS Medium supplemented with	% culture showing shoot formation 1st Week	2nd week	3rd week	4th week	5th week
BAP ($0.5mgL^{-1}$)	8	10	12	15	20
BAP ($1mgL^{-1}$)	10	15	25	34	37
BAP ($1.5mgL^{-1}$)	15	30	40	42	55
IBA ($1.0mgL^{-1}$) + BAP ($1.0mgL^{-1}$)	40	56	71	82	84
BAP ($0.5mgL^{-1}$) + NAA ($0.5mgL^{-1}$)	41	61	72	75	85
BAP ($1.0mgL^{-1}$) + IBA ($2.0mgL^{-1}$)	20	35	40	53	63
BAP ($2.0mgL^{-1}$) + IBA ($1.0mgL^{-1}$)	18	25	35	53	54
BAP ($2.5mgL^{-1}$) + IBA ($1.5mgL^{-1}$)	41	60	72	94	95
BAP ($3.0mgL^{-1}$) + IBA ($1.5mgL^{-1}$)	30	55	68	69	85
BAP ($5.0mgL^{-1}$) + NAA ($2.5mgL^{-1}$)	12	22	34	36	51

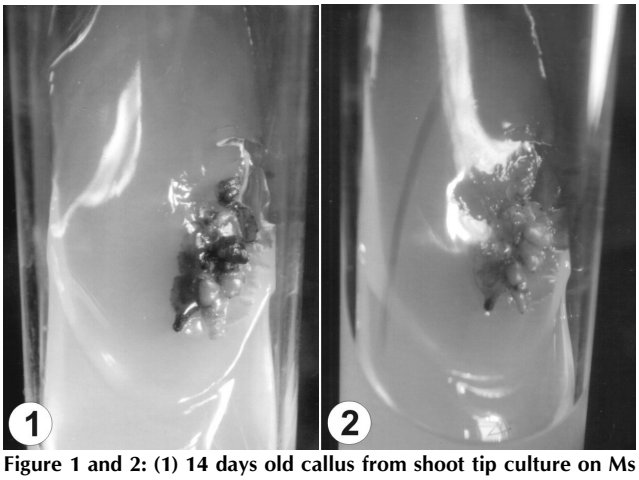

Figure 1 and 2: (1) 14 days old callus from shoot tip culture on Ms medium containing 2, 4-D; (2) 5 weeks old callus from shoot tip on MS medium containing IBA + CW 20% V/V

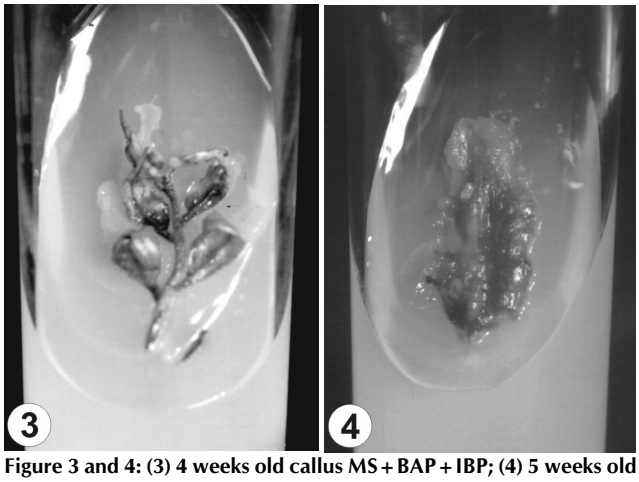

Figure 3 and 4: (3) 4 weeks old callus MS + BAP + IBP; (4) 5 weeks old culture of callus on MS + BAP + NAA

Callus showed a differential response according to the growth regulator used (Table 1 and 2).

The effect of auxin on the induction of callusing from shoot tip and leaf was different. The callus developed was green in colour. The best result was observed at $2.5mgL^{-1}$ concentration of auxin (Fig. 1). Hundred percent cultures exhibited callusing at this concentration. IBA proved to be more effective than IAA in inducing callus from shoot tip, leaf and nodal part of culture. MS medium containing IBA (0.5mg L^{-1} - $3.0mgL^{-1}$), showed callusing from cut end. $2.5mgL^{-1}$ was recorded as the best concentration for callus induction and 100% exhibited callusing (Fig. 2; Table 1).

When MS medium was supplemented with 2.0 mgL^{-1} concentration of cytokinin (BAP). Differentiation of shoot bud from shoot tips was observed in 50% cultures on MS medium supplemented with 1.5 mgL^{-1}. BAP was found most effective in differentiation of shoot bud in comparison to kinetin.

MS basal medium supplemented with combination of cytokinin and auxin shooting and rooting was observed. Most suitable medium found for shoot formation from shoot tips and leaf was a combination BAP (2.5 mgL^{-1}) + IBA (1.5 mgL^{-1}). 95% cultures exhibited shoot differentiation in above combination (Fig. 3 and 4). The better shoot organogenic response has been reported by various workers in different

plant species (Saxena *et al.,* 1998; Zhao *et al.*, 1993). Many authors reported that several plants species highly responded on MS + auxin and Cytokinin combination Yadav *et al*. (1990). High frequency and direct plant regeneration from leaf internode and root segment transformation was found in *Hibiscus sabdariffa* L. (Yadav *et al.*, 2009).

In this experiment, the response microcutting cultured on IBA containing medium was found to be better than that cultured on medium containing NAA. The superiority of IBA over other auxin have also been reported for other tropical fruit trees like Guava (Jaiswal and Amin, 1987), Jack fruit (Amin *et al.*, 1992) and Pummelo (Amin and Akhter, 1993).

In conclusion, this study clearly demonstrated that rapid *in vitro* propagation of *Murraya Koenigii* Sperng can be obtained by proper manipulation of explant age and concentration of plant growth regulators.

REFERENCES

Amin, M.N. and Akhter, S. 1993. Regeneration of plant *in vitro* from seedling explants of pummelos (*Citrus grandis L*. Obs.) *Plant Tissue Cult.* **3:** 71 - 99.

Amin, M. N., Razzaque, M. A. and Akhter, S. 1992. Axillary shoot proliferation and adventitious rooting in vitro of Carambola (*Averrhoa carambola* L.) *Plant Tissue Cult.* **2:** 7 - 13.

Jaiswal, V. S. and Amin, M. N. 1987. *In vitro* propagation of guava from shoot cultures of nature trees. *J. Pl. Physiol.* **130:** 7 - 12.

Jayaweera, D. M. A. 1982. Medicinal plants (Indigenous and Exotic) used in ceylon, The National Science Council of Sri Lanka, Colombo.

Khan, B. A. A., Abraham, A. and Leelamma, S. 1996. Biochemical response in rats to the addition of curry leaf (*Murraya koenigii*) and mustard seeds (*Brassica juncea*) to the diet. *Plants Food for Human Nutrition*. **49:** 295 - 299.

Khan, B. A. A., Abrhana, A. and Leelamma, S. 1998. Role of *Murraya koenigii* and *Brasica juncea* on rats fed antherogenic diet. *J. Food Science and Technology*. **35:** 66 - 68.

Mureshige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassat with tobacco tissue culture. *Physiol. Plant*. **13:** 473 - 497.

Roussos, P. A., Tolia - Morioli, A., Pontikis, C. A. and Kotsias, D. 1999. Rapid multiplication of Jojoba seedling by in vitro culture. *Plant Cell. Tiss. Org. Cult.* **57:** 133 - 137.

Saxena, C., Rout, G. R. and Das, P. 1998. Micropropogation of *Psoralea coryligolia*. *J. Medicinal and Arotnatic Plant Sci.* **20:** 15 - 18.

Xie, J. T., Chang, W. T., Mehendale and Sangeeta, R. 2006. Curry leaf (*Murraya Koenigii Soreng*) reduces blood cholestrol and blood glucose levels in ob/ob mice. *The Amer. J. Chinese Medicine.* **34:** 279 - 284.

Yadav, R., Arora, P., Kumar, D., Kalyal, D., Dilbaghi, N. and Choudhary, A. 2009. High frequency direct plant regeneration from leaf internode and root segments transformation of *Hisbcus sobdariffa* L. *African J. Biotechnology*. **3:** 226 - 228.

Yadav, U., Lal, M. and Jaiswal, V.S. 1990. *in vitro* micropropagation of the tropical fruit tree *syzygium cumini* L. Plant cell. *Tissue and organ cult.* **21:** 87 - 92.

Zhao, Y. X., Yao, D. Y. and Harris P. J. C. 1993. Plant regeneration from callus and explants of *Sesbania spp*. *Plant Cell. Tissue Organ. Cult.* **34:** 253 - 260.