Studying regeneration behaviour of M.emerginata in vitro

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Abstract- In present study cotyledonary nodal segments and nodal shoot segments were used for regeneration. Nodal segment explant as well as cotyledonary nodal explant were incubated on basal MS medium and showed very poor percentage of shooting, explants inoculated on MS medium supplemented with either BAP (0.1-3.0 mg/L) or Kn (0.1-3.0 mg/L) alone produced multiple shoots. Best response of multiple shoot induction was obtained on MS medium supplemented with both BAP (2.0 mg/L) and NAA (0.5 mg/L) and the least responsive combination for multiple shooting in nodal segment as well as cotyledonary nodal explant was 0.1 mg/L Kn. MS medium supplemented with cytokinins alone (BAP or Kn) produced shoots in both the explants but MS medium supplemented with cytokinins (BAP or Kn) + auxins (NAA or IBA) both, produced more percent of shoot regeneration as well as number of shoots / culture. Increased rate of multiplication or shoot induction in cultures supplemented with cytokinin (BAP or KN) and auxin (NAA or IBA) may be due to enhanced rate of cell division by cytokinin (BAP and Kn).

Index Terms- Maytenus emerginata, Cotyledonary nodal segments, Nodal segment explant, cytokinins, auxin

I. INTRODUCTION

Maytenus emerginata **(Willd.)** is an ever green tree that tolerates various types of stresses of the desert and is found in drier parts of central, south –western and north western India. ***Maytenus*** have been used for fever, asthma, rheumatism and gastrointestinal disorders, carcenoma and leukemia, gastrointestinal troubles etc. Medicinal plants are rich source of secondary metabolites, biosynthetically derived fromprimary metabolites but restricted to specific taxonomic genera of plant kingdom and specific part of plant body. Secondary plant products are of major interest because of their biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological and pharmaceutical.

Micropropagation of various plant species including many medicinal plants has been reported (Murashige,1978).Plant regeneration from shoot and stem meristems has yielded encouraging results in medicinal plants like ***Catharanthus roseus, Cinchona ledgeriana, Digitalis spp., Rehmannia glutinosa, Rauvolfia serpentina and Isoplexis canariensis (***Perez-Bermudez *et al.*,2002). Numerous factors are reported to influence the success of ***in vitro*** propagation of different medicinal plants (Short *et al*., 1991). The effects of auxins and cytokinins on shoot multiplication of various medicinal plants have been reported. (Benjamin *et al*., 1987) has shown that 6-Benzylaminopurine (BAP), at high concentration (1-5ppm), stimulates the development of the axillary meristems and shoots tips of ***Atropa belladonna***. (Lal *et al*., 1996) observed a rapid proliferation rate in ***Picrorhiza kurroa*** using kinetin at 1.0-5.0 mg/L. Direct plantlet regeneration from male inflorescences of medicinal Yam on medium supplemented with 13.94 mM kinetin has also been reported (Borthakur and Singh, 2002). The highest shoot multiplication of ***Nothapodytes foetida*** was achieved on medium containing Thidiazuron (TDZ) at a concentration of 2.2 mM by Rai (2002). Barna and Wakhlu (1988) have indicated that the production of multiple shoots is higher in ***Plantage ovata*** on a medium having 4-6 mM Kn along with 0.05 mM NAA.

II. MATERIAL AND METHOD

Cotyledonary nodal segments from ***in vitro*** germinated seedling and nodal shoot segments (2.0 – 3 cm long and 2-4 mm thick) with 1 to 2 nodes plant of ***M. emerginata*** were used for regeneration. The nodal shoot segments were washed with water and then washed with 1.0% solution of Twene -80. The nodal segment explants were washed thoroughly and the detergent was completely washed out. To prevent from fungal contamination nodal segment explants were treated with 6% Bavistin solution and then treated with 70% ethanol which was then followed by 0.1 % $HgCl_2$ for 2-3 minutes in a laminar air flow chamber. These were then washed with autoclaved distilled water for 3-4 times. Prior to inoculation on various media, the surface sterilized explants were then treated with aqueous antioxidant solution (ascorbic acid 50mg/L and citric acid 25mg/L). Cotyledonary nodal segments and sterilized seeds were germinated in the sterilized test tubes containing Paper Bridge. First cotyledonary nodal segments formed after seed germination were used as explants for regeneration. The explants were then cultured on MS medium supplemented with various concentrations of the growth regulators BAP, Kinetin, NAA and IBA, separately or in combination. The pH of the medium was adjusted to 5.8 $\pm$1.0 before autoclaving.

Bud Breaking and Multiple Shoot Induction: Sterilized explants were inoculated horizontally on various media viz, full three fourth and half strength of MS basal salts for culture initiation. Various cytokinins (Kn and BAP) in the range of 0.5-5mg/L were treated for bud breaking and multiple shoot induction. The cultures were then incubated at temperature ranging from 25 to 35°C, 3000 lux light intensity and 60% relative humidity.

Multiplication Of Shoot Cultures: ***In vitro*** regenerated shoots were cut into shoot segments each with one or two nodes and sub cultured on MS basal medium supplemented with

different auxins IAA, NAA (0.01-1mg/L) and cytokinins BAP, Kn (0.25-2.5mg/L) in different concentrations for further multiplication of the shoots. Different media namely half, one-fourth and full strength of MS basal salts were also tested and shoots were also subcultured on media without any PGR. The additives namely ascorbic acid (50mg/L) and citric acid (25mg/L) each were added in the shoot induction as well as in the shoot multiplication media, as antioxidants.

III. RESULTS AND DISCUSSION

The mature nodal shoot segments from field grown plant and cotyledonary nodal segment from ***in vitro*** germinated seedling were exploited for the regeneration of shoots in ***M. emarginata.*** The nodal shoot segment explants collected during months of October-January required longer treatment of mercuric chloride for surface sterilization. On the other hand fresh sprout produced during March-April or July-August found to be more responsive (85%), the bud breaking was observed after 10-12 days of inoculation.

Nodal segment explant as well as cotyledonary nodal explant incubated on basal MS medium, showed very poor percentage of shooting (2 to 5 %). Explants inoculated on MS medium supplemented with either BAP (0.1-3.0 mg/L) or Kn (0.1-3.0 mg/L) alone produced multiple shoots but response was upto 10 % - 15 %.

The best response of multiple shoot induction was obtained on MS medium supplemented with both BAP (2.0 mg/L) and NAA (0.5 mg/L), where 20% to 21% culture produced multiple shoots. The least responsive combination for multiple shooting in nodal segment as well as cotyledonary nodal explant was 0.1 mg/L Kn and multiple shoots obtained were only 3 % and 4 % respectively, both explants were observed to produce least number of shoots on 0.1 mg/L Kn.

MS medium supplemented with BAP (0.1 - 3.0 mg/L) alone showed variation in the induction of multiple shoots which ranged between 5 % to12 % in nodal segment explant and 6 % to 14 % in cotyledonary nodal explant, while Kinetin alone (0.1-3.0 mg/L) it ranged from 3 % to 25 % and 4 % to 9 % respectively.

MS medium supplemented with cytokinins alone (BAP or Kn) produced shoots in both the explants but MS medium supplemented with cytokinins (BAP or Kn) + auxins (NAA or IBA) both, produced more percent of shoot regeneration as well as number of shoots / culture. NAA in combination with BAP and Kn showed better response than IBA with BAP and Kn. Average shoots / culture (1.5 and 1.6) as well as average length of shoots (1.7 and 1.8 cm) were maximum in MS medium supplemented with BAP (2.0 mg/L) + NAA (0.5 mg/L) from ***in vitro*** nodal segment and ***in vitro*** cotyledonary nodal segment respectively. Increased rate of multiplication or shoot induction in cultures supplemented with cytokinin (BAP or KN) and auxin (NAA or IBA) may be due to enhanced rate of cell division by cytokinin (BAP and Kn).

Table .1
IN VITRO* SHOOT INDUCTION IN *M. emarginata

Growth regulators (mg/L)	**% Shoot regeneration**		**Average shoots per culture**		**Average length of shoots (cm)**	
	***In vivo* nodal segment**	***In vitro* cotyledonary nodal segment**	***In vivo* nodal segment**	***In vitro* cotyledonary nodal segment**	***In vivo* nodal segment**	***In vitro* cotyledonary nodal segment**
BAP						
0.1	5	6	0.3	0.4	0.4	0.4
0.5	5	6	0.5	0.4	0.6	0.6
1.0	7	8	0.8	0.9	0.9	1.0
1.5	9	11	1.1	1.2	1.1	1.3
2.0	12	14	1.3	1.5	1.5	1.6
2.5	8	10	0.9	0.9	1.0	0.9
3.0	6	7	0.7	0.7	0.7	0.7
Kinetin						
0.1	3	4	0.2	0.2	0.3	0.4
0.5	4	5	0.3	0.3	0.4	0.5
1.0	5	6	0.5	0.5	0.5	0.6
1.5	7	8	0.7	0.8	0.6	0.8
2.0	8	9	0.9	1.1	0.9	1.1
2.5	7	7	0.6	0.7	0.5	0.9
3.0	5	5	0.4	0.5	0.3	0.6
BAP+NAA						
0.1+0.1	10	11	0.3	0.4	0.5	0.5
0.5+0.2	11	13	0.5	0.6	0.6	0.6
1.0+0.3	13	15	0.8	0.9	0.9	1.0
1.5+0.4	16	18	1.2	1.3	1.3	1.4
2.0+0.5	**20**	**21**	**1.5**	**1.6**	**1.7**	**1.8**

2.5+0.6	15	17	1.0	1.1	1.2	1.3
3.0+0.7	11	12	0.7	0.8	0.8	0.9
BAP+IBA						
0.1+0.1	8	8	0.2	0.2	0.3	0.3
0.5+0.2	9	9	0.3	0.3	0.5	0.5
1.0+0.3	11	12	0.5	0.6	0.7	0.8
1.5+0.4	14	15	0.7	0.9	1.0	1.1
2.0+0.5	16	18	1.0	1.1	1.3	1.4
2.5+0.6	13	14	0.6	0.8	0.9	1.0
3.0+0.7	10	11	0.4	0.5	0.6	0.7
Kn+NAA						
0.1+0.1	8	8	0.3	0.3	0.4	0.4
0.5+0.2	9	9	0.4	0.4	0.6	0.6
1.0+0.3	11	12	0.6	0.7	0.8	0.9
1.5+0.4	13	14	0.8	0.9	1.0	1.1
2.0+0.5	15	16	1.0	1.2	1.3	1.5
2.5+0.6	12	13	0.7	0.8	0.9	1.0
3.0+0.7	8	9	0.5	0.5	0.6	0.6
Kn+IBA						
0.1+0.1	4	4	0.2	0.2	0.2	0.3
0.5+0.2	5	5	0.3	0.3	0.4	0.4
1.0+0.3	6	7	0.4	0.5	0.6	0.7
1.5+0.4	8	9	0.6	0.7	0.8	0.9
2.0+0.5	10	11	0.8	0.9	1.0	1.1
2.5+0.6	7	8	0.4	0.5	0.6	0.7
3.0+0.7	5	5	0.2	0.3	0.3	0.5

IV. CONCLUSION

Micropropagation has a number of advantages over traditional plant propagation techniques. Micropropagation produces disease free plants and has an extraordinarily high fecundity, producing thousands of propagules in the same time. It can be a conventional technique to produce hundreds of plants, genetically identical with the donor or parent plants. Micropropagation produces rooted plantlets ready for growth rather than seeds or cuttings. It produces more robust plants, leading to accelerated growth compared to similar plants produced by conventional methods. ***In vitro*** propogation of plants holds tremendous potential for the production of high quality plant based medicines .Hence micropropagation of ***M. emerginata can*** be a achievement in the field of Biotechnology.

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