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Factors Affecting Micropropagation of Maytenus Emarginata: A Plant of Stressed Ecosystem

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Abstract: ***Types of media (MS basal, MS three- fourth, MS half, B_5 and WP), explants and growth regulators (auxin, cytokinin) with various concentration played important role in the bud breaking or multiple shoot induction under in vitro. Out of various media and growth regulators tested the maximum number of explants were responded on MS full strength medium supplemented with NAA 0.1 mg/l + BAP 5.0 mg/l +additives. Different type of cytokinin (2-IP, KN, BAP) with concentration from 0.25-2.5 mg/l) were tested but best result from subcultured shoot were found on MS medium supplemented with IAA 0.1 mg/l + BAP 2.5 mg/l+ additives (ascorbic acid 50 mg/l, citric acid 25 mg/l L-arginine 25 mg/l and adenine sulphate 25 mg/l). On this medium the nodal shoot segment explants which were taken from lopped and managed tree were found the best for shoot induction. These cultures were incubated at 28±2 C under regime of 12 h photoperiod of light for shoot induction.***

Keywords: in vitro, medium, subculture, inoculation, explant

1. Introduction

Maytenus emarginata (Celastraceae) is a valued tree of Indian desert. This plant stabilizes the soil and provides fodder and fuel. The plant yields timber and it has medicinal value [Bhandari, 1990]. Though its timber is not utilized commercially but it is very heavy, and it has polishing quality. It is used for making durable agriculture appliances. Besides this, it is ecologically very important as soil stabilizer. It provides protection to microbe, birds and small annual and perennial plants like Ephedra foliate which is threatened plant. This plant shows great variability in several traits like plant height, bole character, and quality of timber. This is because the plant is out breeder.

Maytenus emarginata (Wild.) Ding-Hou, is a highly drought resistant biomass producer of arid region. During recent years ruthless cutting has resulted in disappearance of valuable germplasm from the arid and semi-arid regions. Since no conventional method of vegetative propagation is available, therefore alternative techniques must be applied to multiply the germplasm with superior traits like fast growth and resistant to salinity and high temperature (Ahuja et al.1993, Gupta et al 1989, Hammatt 1992).

2. Materials and Methods

Extensive field survey of arid and semi-arid region i.e. Barmer, Jaisalmer, Jodhpur, Sikar, Churu and Pali were explored in order to select mother plant (for collection of explants).The explants were collected from different lopped and unlopped plant during all season. Various type of explants viz. nodal shoot segments, internodal segments, leaf and root were tested for shoot induction.

These explants were taken to laboratory and wash with tween 80 followed by running tap water. These explants were surface sterilized with 70-90% of ethanol for 60-90 sec followed by 0.1 % of, mercuric chloride for 4-7minutes. The steriliant treated explants were washed 4-6 times with sterile distilled water and inoculated either vertically or horizontally on culture medium.

These surface sterilized explants were inoculated on different types of media viz. B5 (Gamborg et al), WP, Ms-full strength, Ms half strength and MS three- fourth strength for shoot buds' proliferation. Various experiments were conducted to know the effect of cytokinin (KN, 2- iP. BAP) with concentration of 0.25-2.5mg/l+ additives on shoot bud proliferation. After harvesting differentiated shoots from nodal region of explants, the mother explants were repeatedly transferred on fresh MS medium supplemented with0.1mg/l of IAA and different concentration of BAP (1.0-5.0 mg/l) along with additives to yield fresh crop of shoot.

3. Results and Discussion

The surface sterilized explants were inoculated on difference type of media with NAA 0.1 mg/l +BAP 5.0 mg/l + additives (Table 1). The highest number of explants i.e., 75-80% were responded on MS (Murashige and Skoog) full strength basal medium supplemented with 0.1 mg/l NAA + 5.0 mg/l BAP. On this medium maximum 8-10 shoot per node were produced, while lesser number of shoots i.e., less than 5 were produced on other media. B_5 medium was found to be less effective for shoot bud induction. Out of various type of explants tested, the nodal shoot segments which were taken from lopped tree were found the best for shoot induction (Table 2). The mother explants were reused 4 to 6 times to get fresh crop of shoot under in vitro.

Various explants viz. shoot segment nodal shoot segment, leaf and roots were tested for callus induction. Shoot segment found to be the best explants for callus induction followed by leaf. Various combination of BAP + 2,4- D and kinetin + NAA were tested for callus induction under in vitro condition. Best result was found on BAP 0.1 mg/l + 2,4-D 1.0 mg/l (Table-1). Tissue grows rapidly on this medium and produced cremish white, shiny, and soft callus.

In vitro grown shoots were taken for further subculture. These shoots were cultured on MS medium supplemented with different types of cytokinin (KN, 2 -iP, BAP) with various concentration (0.25-2.5 mg/l). The highest number of shoots were produced i.e., 6-8 shoot per node on MS medium containing 0.1 mg/l IAA + 2.5 mg/l BAP +additives (Table 3). The kinetin and 2-iP was found less effective for shoot bud proliferation. The various concentration of BAP (1.0-5.0mg/l) along with 0.1 mg/l of IAA were tested for shoot induction. It was found that on higher concentration of BAP although maximum number of shoot induction were occurred but shoot remain dwarf. In many desert plants, the tissue culture methodology is available for mass multiplication. (Deora et al 1995, Shekhawat et al 1993, Rathore et al 1993). Each experiment was consisted of fifteen replicate and repeated three times. These cultures were incubated at 28±2 C under 12h of photoperiod (4000-4500 lux intensity of light).

Abbreviation; BAP-6 benzyleaminopurine, NAA-nepthalene acetic acid,2-iP-iso- pentenyl adenine, IAA-indole-3-acetic acid, SD-standard deviation, WP-woody plant medium,

Table 1: Effect of various media in multiple shoot induction from nodal portion of explant of M.emarginata after 3 weeks

Media Used*	*Explants responded ± SD*	*Shoot number/ explants ± SD*	*Shoot length (length)± SD*
B_5	56.0±4.2	3.2±0.8	1.6±0.4
WP	62.0±5.7	3.4±0.8	1.7±0.6
½ MS	65.0±3.5	3.4±1.1	2.0±0.6
¾ MS	69.0±4.2	5.2±0.8	2.2±0.6
MS	76.0±5.2	7.2±1.3	2.76±0.3

Media supplemented with NAA 0.1 mg/l + BAP 5.0 mg/l + additives

Table 2: The shoot induction from different types of explants of M. emarginata on MS medium supplemented with 0.1 mgl^{-1} IAA + 2.5 mgl^{-1} BAP + additives.

Explant type	*Percentage of explants responded ± SD*	*Number of shoots per node ± SD*	*Shoot length (cm) ± SD*
Nodal shoot segment (unlopped tree)	66.4±2.6	6.6±0.9	2.1±0.5
Nodal shoot segment (lopped tree)	80.2±2.4	11.2±2.6	2.4±0.4
Apical shoot segment	66.8±1.9	7.4±0.4	1.9±0.3

Table 3: Effect of various cytokinins on shoot multiplication from subcultured shoots of M.emarginata on MS medium containing MS+IAA 0.1 mg/l + additives.

Cytokinin (mg/l)	*Shoot numbers per explant ± SD*	*Shoot length (cm) ± SD*
Control	1.4±0.6	1.6±0.4
2- iP		
0.25	1.6±0.6	1.8± 0.3
0.5	2.4±0.6	1.9±0.4
1.0	3.6±0.6	2.6±0.4
2.5	4.4±0.8	1.7±0.3
KN		
0.25	1.6±0.6	2.8±0.8
0.5	2.6±0.6	2.1±0.4
1.0	3.6±0.6	1.9±0.4
2.5	5.0±0.7	0.9±0.4
BAP		
0.25	1.8±0.8	2.9±0.4
0.5	3.2±0.8	2.4±0.4
1.0	4.8±0.8	2.2±0.6
2.5	6.0±1.2	0.9±0.4

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