Efficient Micropropagtion Protocol for *Portulaca grandiflora*. Hook. Using Shoot Tip Explants

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Abstract: A rapid clonal multiplication protocol comprising direct multiple shoot induction from shoot apex of *Portulaca grandiflora* Hook was developed. Shoot apex from healthy grown plants were used as explants for culturing. Explants were cultured on standard Murashige and Skoog (MS) medium supplemented with different concentrations of benzyl amino purine (BAP) or kinetin (KIN) for primary shoot proliferation. The best shoot proliferation (27.3 per explants with 98% induction) was observed in MS medium containing 2.5mgl^{-1} BAP. For rooting of microshoots, half strength MS medium supplemented with 0.75mgl^{-1} Naphthalene acetic acid (NAA) showed best results with 9.2 roots per shoot at an average root length of 6.0 cm with average rooting response of 95%. After acclimatization and transplantation, 100% of the *In- vitro* derived plants were found healthy in *ex vivo* conditions. [New York Science Journal. 2010;3(10):112-116]. (ISSN: 1554-0200).

Key word: Acclimatization, *Portulaca grandiflora* Hook, *In-vitro*, Shoot proliferation.

1. Introduction

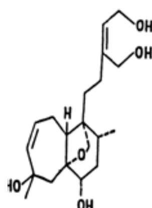
Medicinal plants have been the subject of curiosity since times immemorial (Constable, 1990). Almost every civilization has a history of uses of medicinal plants. Approximately 80% of the people in the world of developing countries rely on the traditional medicines for their primary health care needs and about 85% of traditional medicines involve the use of plant extract. The resurgence of public interest in plant based medicine coupled with rapid expansion of pharmaceutical industries has increased demand of medicinal plants; as a consequence, the rates of exploitation may exceed those of local natural regeneration practices. There is thus an urgent need to develop and impelement regeneration and conservation strategies for sustainable exploitation of these important medicinal plant species. The common means of regeneration and proliferation of medicinal plants include micropropagtion methods. The most important technique in micropropagtion is meristem proliferation wherein apical or nodal segments harbouring an axillary bud are cultured to regenerate multiple shoots without any intervening callus phase.

In-vitro propagation has revolutionized commercial nursery business (Pierik, 1991). Significant features of *in-vitro* propagation procedure are its enormous multiplicative capacity in a relatively short span of time; production of healthy and disease free plants; and its ability to generate propagules around the year (Dhawan and Bhojwani, 1986). *In-Vitro* propagation of medicinal plant species hold tremendous potential for the production of high quality plant based medicines (Murch *et al.,* 2000). The availibity of the shoot cultures throughout the year and the circumvention of surface sterilizing procedures with greater assurance of non-contaminated cultures make them ideal source for various purposes. Micropropagated plants can be tested or sold with the assurance that they are genetically uniform.

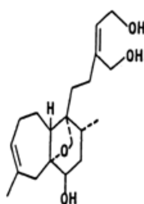
Portulaca grandiflora Hook. (Portulacaceae) commonly called as Moss rose in English, Nonia in Hindi, Pung mapan satpi in Manipuri and Gul-e-Shama in Urdu, is a succulent plant, profusely branched approximately 10-30cm high, leaves about 12-35 mm in length and 1- 4 mm in width, linear-subulate, thick, fleshy and spirally arranged. Petiole is mostly short with axillary hairs. Its colorful flowers are 2-3cm across with conspicuous stamens and arranged in a terminal capitulum, surrounded by whorls of leaves. Two sepals are about 6mm long with a very small apical keel. Petals are broadly obovate, deeply notched and posses red, pink, or purple colours. Fruits are subglobose and 4-6mm in diameter. Seeds are very small and shining.

The plant is used for the cure of sore throat and skin rashes. It is a putative immunostimulant (Chavalittumrong *et al*., 2007). It is also used for detoxification. It has also been reported for its

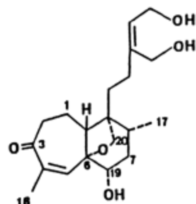
efficacy on hepatitis B surface antigen (Zheng and Zhang, 1990). In addition, antimutagenic effect on the mutation induced by alfatoxin B1 and cyclophosphamide in mice has been demonstrated (Liu *et al*., 1990). Aerial parts are reported to contain various diterpenoids like portulal, portulenone, portulenol, portulene (Ohasaki *et al*., 1986) and portulene acetal a minor diterpenoid (Ohasaki *et al*., 1997).



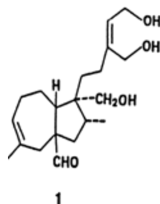
Portulenol



Portulene



Portulenone



Portulal

During the present investigations, in an endeavor to develop micropropagtion protocol, efficient plant regeneration was obtained using shoot apex as explants. The protocol could go a long way in the further biotechnological improvement and commercial exploitation of this important plant species.

2. Material and Methods

A successful micropropagation protocol proceeds through a series of stages each with specific set of requirement and proper precautions. These are (i) initiation of aseptic cultures (ii) Shoot multiplication (iii) rooting of microshoots (iv) hardening and field transfer of *in-vitro* grown plantlets. Plants of *Portulaca grandiflora* were collected from the botanical garden of School of Studies in Botany Jiwaji University Gwalior, M.P, India. Shoot apex taken from the actively growing shoots were washed for 10 minutes under continuous stream of running tap water. Explants were surface sterilized with 0.1% (w/v) $HgCl_2$ for 3 minutes and then rinsed four times with autoclaved double distilled water under aseptic conditions in laminar chamber. 25 ml of the molten (MS) basal medium (Murashige and Skoog 1962) supplemented with different concentrations (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0) of 6-benzylaminopurine (BAP) or Kinetin (KIN) was dispensed into culture tubes (25×150mm), plugged with non-adsorbent cotton. The excised explants 0.5 cm in length were inoculated in these culture tubes for shoot proliferation. Individual regenerated shoots (3-4 cm long), were excised from the shoot clumps and transferred to half strength MS medium containing various concentrations of IBA or NAA for root induction. All the media contained 3% sucrose (w/v) as carbon source. Media were adjusted to pH 5.7 using 0.1N NaOH or 0.1N HCl before addition of 0.8% agar (w/v) and autoclaved at 121^oC for 15 min. The cultures were incubated at 25± 2^oC and 70-80% relative humidity under a 16-h Photon flux density provided by cool white fluorescent light. The rooted microshoots were transferred to pots filled with a mixture of well sterile soil: sand: vermiculite in the ratio of 1:1:1 (v/v). After proper hardening the plants were transferred to field condition with 100% survival.

3. Results and Discussion

Proliferation of multiple shoots was observed with high frequency from shoot tips with twelve days of inoculation. These explants were capable of directly developing multiple shoots on MS medium containing different concentrations of BAP or Kinetin (Table- 1 & Figures- 1, 2, 3). The highest percentage of shoot induction was 98.0±3.5% on the medium augmented with BAP 2.5mgl^{-1} (Table- 1) followed by 90.3±2.3% on the medium supplemented with 3.0mgl^{-1} BAP. On the other hand the lowest percentage of multiple shoot induction was found to be 52.0±1.2% on MS medium with KIN 3.5mgl^{-1}. Maximum number of 27.3±3.5 shoots per explants with an average length of 5.5 ± 0.9 cm were obtained on the MS medium with 2.5 mgl^{-1} BAP.

Continued exposure of explants to concentrations higher than 2.5 mgl^{-1} BAP during shoot induction caused high accumulation of cytokinin which inhibited further shoots from developing. (Bhau and Wakhlu 2003) observed that high concentration of BAP resulted in decrease in shoot multiplication rate of mulberry. High concentrations of cytokinin also have been reported for the reduction of shoot-bud induction frequency in *Bacopa monniera* nodal explants (Tiwari *et al*.,2001). A total of 20.6±2.5 shoots per explants with an average length of 4.8±0.2cm were obtained on media forfeited with KIN 2.0 mgl^{-1}. BAP showed better results as compared to KIN (Table-1), which is in similarity with the earlier reports on *Azadirachta indica* (Arora *et al*., 2010) *Zingiber officinale* (Balachandran *et al*.1990), *Houttuynia cordata* (

Handique and Bora, 1999) , *Sida cordifolia* (Sivanesan *et al*., 2007) *Morus alba* (Balakrishnan *et al*., 2009) and *Podophyllum hexandrum* (Chakraborty *et al*.,2010). The concentration of BAP higher than 4.0 resulted in callus induction (Figure-4. Data not Shown). The callus was creamy colored and of soft texture.

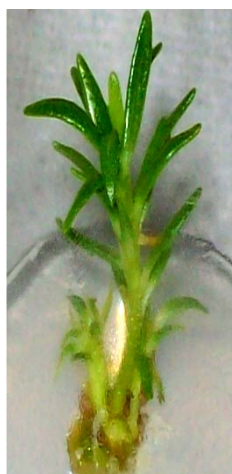


Figure-1.

Figure-2

Figure-3

Figure-4

Fig 1. Direct Shoot regeneration
Fig 2. Multiple shoot induction
Fig 3. Shoot elongation
Fig 4. Callus inductin

Table 1. Effect of BAP or KIN on shoot induction from shoot apex of *Portulaca grandiflora* after two weeks of culturing.

MS media+ Growth Harmone	Conc. (mgl^{-1})	Shoot induction rate %	No of shoots / explant	Average Shoot length (cm)
BAP	0.00	0.00	0.00	0.00
	0.5	65.3 ± 1.7	7.50 ± 1.3	4.2 ± 0.7
	1.0	75.1 ± 2.3	9.30 ± 2.8	4.5 ±1.3
	1.5	83.0 ± 2.7	13.4 ± 1.5	4.0 ±1.0
	2.0	87.6 ± 3.0	22.4 ± 2.1	5.2 ± 0.5
	2.5	**98.0 ± 3.5**	**27.3 ± 3.5**	**5.5 ± 0.9**
	3.0	90.3 ± 2.3	20.2 ± 2.7	4.1 ±1.2
	3.5	90.0 ± 2.5	17.3 ± 0.9	4.9 ± 0.8
	4.0	90.0 ± 1.9	15.5 ± 1.4	4.6 ± 0.5
KIN	0.00	0.00	0.00	0 .00
	0.5	70.0 ± 0.0	3.0 ± 0.0	3.9 ±0.3
	1.0	70 .5 ± 0.5	7.0 ±0.00	4.5 ±0.7
	1.5	78.0 ± 1.0	12.3 ± 0.6	4.5 ±1.2
	2.0	**80.5 ± 1.5**	**20.6 ± 2.5**	4.8 ±0.2
	2.5	77. 5 ± 1.5	18.3 ± 2.9	3.8 ± 0.1
	3.0	73.0 ± 3.5	17.0 ±0.7	4.1 ±0.6
	3.5	52.0 ± 1.2	15.0± 0.5	4.6 ± 0.5

Values are Mean ± S.D based on 20 replicates per treatment

For rooting, shootlets when cultured on auxin free half strength MS medium elicit mild root proliferation 10±0.2% with a maximum of 2.2±0.3 roots per shoot at an average length of 4.2cm. The possible reason for this may be the endogeneous pool of auxins synthesized in the apical portion of the shootlets and its downward translocation. Microshoots transferred to half strength MS media supplemented with different concentrations of NAA or IBA rooted well (Table-2 & Figure-5).

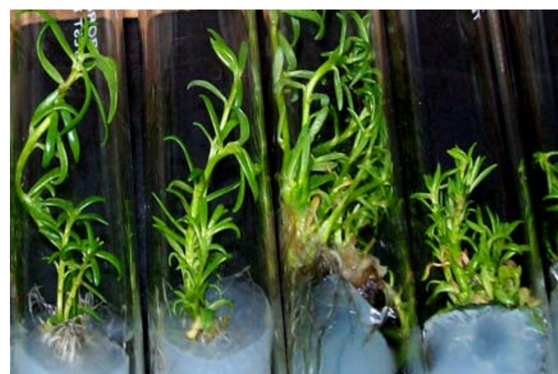


Figure -5 Plantlets grown on half strenght MS Media containing 0.75 NAA for rooting

Better root initiation (95%) and development 9.2 roots per shoot with an average length of 6.0 cm was observed on ½ MS supplemented with $0.75 mgL^{-1.}$ NAA .The superiority of NAA over IBA is in conformity with the earlier results of Sivanesan and Jeong (2009).

Table 2. Effect of auxins (NAA & IBA) on *in vitro* rooting after 25 days of culture.

Growth Regulator	Conc. (mgl^{-1})	Rooting Response (%)	roots/shoot	Root length (cm)
	0.00	10±0.7	2.2±0.3	4.2±0.4
NAA				
	0.5	80 ± 1.2	6.4 ± 0.6	4.9 ± 0.2
	0.75	95 ± 2.0	9.2 ± 1.5	6.0 ± 0.6
	1.0	90 ± 2.5	7.5 ± 0.8	4.5 ±1.0
IBA				
	0.5	78 ± 0.5	6.5 ± 0.5	3.5 ± 0.1
	1.0	70 ± 1.3	5.4 ± 1.0	4.5 ± 0.7

Values are Mean ± S.D based on 20 replicates per treatment

The successful acclimatization of micropropagated plants and their subsequent transfer to the field is a crucial step for commercial exploitation of *in vitro* technology. The rapid desiccation of *in vitro* grown plantlets and their susceptibility to diseases due to high humidity are major obstacles in their successful acclimatization. In the present investigation all the regenerants acclimatized well in the green house and then in outdoor conditions (Figure-6&7)

Figure-6

Figure-7

Survival rate of 100% was achieved when the rooted shoots were transferred to pots containing soil, sand and Vermiculite in the proportion of 1:1:1 and irrigated with tap water. Same combination of pot mixture were used to raise the regenerated shoots of *A. vera-cruz.* (Tejavathi and Gayathramma, 2007). 75% survival rate was achieved when the rooted shoots of *Vigna monga* were transferd to pots containing manure and soil mixtures supplemented with Hoaglands solution (Ignacitmuthu and Franklin 1999), While as in present investigation, tap water irrigation was found enough to achieve 100% survival of regenerated plants.

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