

***In-vitro* Propagation of a Medicinal Plant *Portulaca grandiflora*. Hook**

Ashok K. Jain and Mudasir Bashir

Plant Tissue Culture Laboratory,
 School of Studies in Botany Jiwaji University,
 Gwalior - 474011, Madhya Pradesh, India

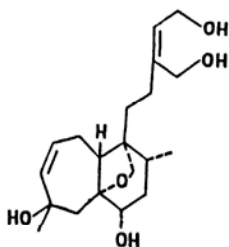
Abstract: Present investigations were carried out with a view to standardize an *in-vitro* culture technique for propagation of *Portulaca grandiflora* Hook. Nodal segments from healthy grown plants were used as explants for culturing. Explants were cultured on standard Murashige and Skoog (MS) medium supplemented with different concentrations of benzyl amino purine (BAP) or kinetin (KIN) for primary shoot proliferation. Best shoot proliferation (56.55 per explants with 98% induction) was observed in MS medium containing 4.0mgL^{-1} BAP. For rooting of the microshoots, half strength MS medium supplemented with 0.75mgL^{-1} Naphthalene acetic acid (NAA) exhibited 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.

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

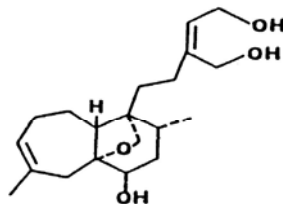
INTRODUCTION

Portulaca grandiflora Hook. (Portulacaceae) is a succulent plant and 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. The petiole is mostly short with axillary hairs. Its colorful flowers are 2-3cm across with conspicuous stamens and arranged in a terminal capitulum which is surrounded by whorls of leaves. Two sepals are about 6mm long with a small apical keel. Petals are broadly obovate, deeply notched and possess red, pink, or purple

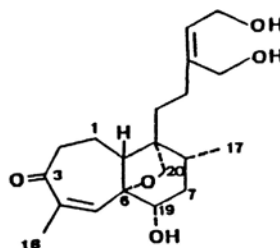
colours. Fruits are subglobose of 4-6mm in diameter. Seeds are very small and shining. The plant is used for the cure of sore throat and skin rashes. It is a putative immunostimulant [1]. It is also used for detoxification. *Portulaca grandiflora* has been reported for its efficacy on hepatitis B surface antigen [2]. In addition, antimutagenic effect on the mutation induced by aflatoxin B1 and cyclophosphamide in mice has been demonstrated [3]. Aerial parts of *Portulaca grandiflora* are reported to contain various diterpenoids like portulal, portulene, portulenol, portulene [4] and portulene acetal a minor diterpenoid [5].



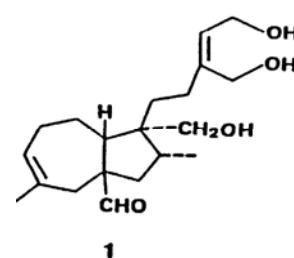
Portulenol



Portulene



Portulenone



Portulal

Nodal shoot proliferation has much potential as supporting technology for micropropagation of ornamental and medicinal plants. The availability 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. For micropropagation of novel ornamental and medicinal plants direct shoot proliferation using nodal explants is a major biotechnological approaches. Micropropagated plants can be tested or sold with the assurance that they are genetically uniform. *In vitro* shoot formation from nodal segments is helpful in producing true to type plants and in maintaining genetic stability of the plants and also in genetic transformation. During the present investigations, in an endeavor to develop micropropagation protocol, efficient plant regeneration was obtained using nodal explants. The protocol could go a long way in the further biotechnological improvement of this important plant species.

MATERIALS AND METHODS

Plants of *Portulaca grandiflora* were collected from the botanical garden of School of studies in Botany Jiwaji University Gwalior, M.P, India. Nodal explants 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 rinsed four times with autoclaved double distilled water under aseptic conditions in laminar chamber.

25 mL⁻¹ of molten (MS) basal medium [6] supplemented with different concentrations (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0) of 6-benzylaminopurine (BAP) or Kinetin (KIN) was dispensed into culture tubes (25×150 mm), plugged with non-adsorbent cotton. The excised nodal 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 indole butyric acid (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°C for 15 min. The cultures were incubated at 25± 2°C 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 and vermiculite in the ratio of 1:1:1 (v/v/v). After proper hardening the plants were transferred to field condition with 100% survival.

RESULT AND DISCUSSION

Cytokinins reported to overcome apical dominance, induce high number of shoot buds and release lateral buds from dormancy. Therefore, MS basal media supplemented with different concentrations of BAP or KIN were used to induce shoot proliferation in the nodal explants of *Portulaca grandiflora* (Table 1 and Fig. 1).

Table 1: Effect of BAP or KIN on shoot induction from nodal explants of *Portulaca grandiflora* after two weeks of culturing

| MS media +Growth Regulator | Concentration (mgL ⁻¹) | Shoot induction rate % | No of shoots per explant | Average length of longest shoot (cm) |
|----------------------------|------------------------------------|------------------------|--------------------------|--------------------------------------|
| BAP | 0.00 | 0.00 | 0.00 | 0.00 |
| | 0.5 | 75.2 ± 2.3 | 9.90 ± 1.0 | 3.46 ± 0.0 |
| | 1.0 | 79.5 ± 1.0 | 14.40 ± 0.3 | 3.75 ± 0.5 |
| | 1.5 | 85.0 ± 2.7 | 19.44 ± 0.3 | 4.34 ± 0.1 |
| | 2.0 | 87.6 ± 3.0 | 27.19 ± 3.5 | 4.14 ± 0.1 |
| | 2.5 | 92.0 ± 2.1 | 32.88 ± 2.1 | 4.38 ± 0.7 |
| | 3.0 | 95.6 ± 2.0 | 39.75 ± 0.7 | 4.70 ± 0.8 |
| | 3.5 | 95.0 ± 1.9 | 41.48 ± 2.3 | 5.23 ± 0.0 |
| | 4.0 | 98.0 ± 0.7 | 56.55 ± 1.3 | 6.50 ± 0.1 |
| KIN | 4.5 | 87.5 ± 3.7 | 38.60 ± 0.1 | 5.35 ± 0.3 |
| | 0.00 | 0.00 | 0.00 | 0.00 |
| | 0.5 | 64 ± 2.6 | 6.0 ± 0.0 | 3.8 ± 0.1 |
| | 1.0 | 68.6 ± 1.5 | 9.0 ± 1.02 | 4.16 ± 0.7 |
| | 1.5 | 72.0 ± 2.0 | 15.3 ± 0.2 | 4.6 ± 0.2 |
| | 2.0 | 75.2 ± 1.5 | 20.6 ± 2.0 | 5.06 ± 0.8 |
| | 2.5 | 80.0 ± 1.9 | 22.3 ± 1.7 | 5.46 ± 1.0 |
| | 3.0 | 86.0 ± 2.3 | 30.7 ± 1.3 | 6.86 ± 0.3 |
| | 3.5 | 80.0 ± 1.7 | 28.0 ± 1.0 | 6.53 ± 0.6 |
| | 4.0 | 76.6 ± 1. | 25.6 ± 2.5 | 5.53 ± 1.3 |

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

| Growth Regulator | Concentration (mgL ⁻¹) | Rooting Response (%) | No. of roots/explant | Root length (cm) |
|------------------|------------------------------------|----------------------|----------------------|------------------|
| NAA | 0.50 | 80 ± 1.2 | 6.4 ± 0.6 | 4.9 ± 0.2 |
| | 0.75 | 95 ± 2.0 | 9.2 ± 1.5 | 6.0 ± 0.6 |
| | 1.00 | 90 ± 2.5 | 7.5 ± 0.8 | 4.5 ± 1.0 |
| IBA | 0.50 | 78 ± 0.5 | 6.5 ± 0.5 | 3.5 ± 0.1 |
| | 1.00 | 70 ± 1.3 | 5.4 ± 1.0 | 4.5 ± 0.7 |

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



Fig. 1: Regeneration of large number of multiple shoots



Fig. 2: Plantlets grown on half strength MS Media containing 0.75 NAA for rooting

Multiple shoots proliferated within two weeks of culture. The highest number of regenerated shoots obtained was 56.55 per explant on medium fortified with BAP 4mgL⁻¹ with an average induction rate of 98%. Concentrations of BAP higher than 4mgL⁻¹ exhibited negative effect on shoot regeneration. Continued exposure of explants to high concentrations of BAP during shoot induction



Fig. 3: An acclimatized plant under natural conditions

caused high accumulation of cytokinin which inhibited development of further shoots. Bhau and Wakhlu [7] observed that high concentration of BAP resulted in a decrease in shoot multiplication rate in mulberry plant. High concentrations of cytokinin have also been reported for the reduction of shoot-bud induction frequency in *Bacopa monniera* [8]. In present observation shoot length was found to be maximum (6.8 cm) in KIN (3mgL⁻¹) supplemented media. BAP showed better results as compared to KIN (Table 1), which is parallel with the earlier reports on *Zingiber officinale* [9], *Houttuynia cordata* [10], *Sida cordifolia* [11], *Morus alba* [12] and *Azadirachta indica* [13].

For rooting, shootlets obtained from repeated cultures were transferred to half strength MS media supplemented with different concentrations of NAA or IBA supplemented individually to the media for rooting (Table 2 & Fig. 2). 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 0.75mgL⁻¹ NAA.

All the regenerants acclimatized well in the green house and then under outdoor conditions (Fig. 3). 100% survival was achieved when the rooted shots 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 *Aloe vera-cruz*. [14]. A survival of 75% was achieved when the rooted shoots of *Vigna mungo* were transferd to pots containing manure and soil mixtures supplemented with Hoaglands solution [15] while in present investigation, Tap water irrigation was found suitable to achieve 100% survival of regenerated plants.

REFERENCES

1. Chavalittumrong, P., B. Sriwanthana, A. Rojanawiwat, R. Kijphati, B. Jitjuk, W. Treesangsi, S. Phadungpat, J. Bansiddhi and M. Bunjob, 2007. Safety of the aqueous extract of *Portulaca grandiflora* Hook in healthy volunteers Songklanakarin J. Sci. Technol., 29(Suppl. 1): 95-100.
2. Zheng, M.S. and Y.Z. Zhang, 1990. Anti-HBsAg herbs employing ELISA technique. Zhong Xi Yi Jie He Za Zhi, 10: 560-562
3. Liu, D., X. Yin, H. Wang, Y. Zhou and Y. Zhang, 1990. Antimutagenicity screening of water extracts from 102 kinds of Chinese medicinal herbs. Zhongguo Zhong Yao Za Zhi, 15: 617-622.
4. Ohsaki, A., K. Shibata, T. Tokoroyama, T. Kubota and H. Naoki, 1986. Novel diterpenes with bicyclo [5.4.0] undecane skeleton from *Portulaca grandiflora* Hook, Possible linking intermediates in the biosynthesis of Portulal. Chemistry Letters., pp: 1585-1588.
5. Ohsaki, A., T. KubotaKozo Shibata and T. Tokoroyama, 1997. Portulene acetal, a novel minor constituent of *Portulaca grandiflora* with significance for the biosynthesis of Portulal. J. Nat. Prod., 60: 912-914.
6. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.
7. Bhau, B.S. and A.K. Wakhlu, 2003. Rapid micropropagation of five cultivars of mulberry. Biol Planta., 46(3): 349-355.
8. Tiwari, V., K.N. Tiwari and B.D. Singh, 2001. Comparative studies of cytokinins on *in vitro* propagation of *Bacopa monniera*. Plant Cell Tiss Org. Cult., 66: 9-16.
9. Balachandran, S.M. and S.R. Bhat, 1990. *In vitro* clonal multiplication of turmeric (*Curcuma* Spp.) and ginger (*Zingiber officinale* Rose). Plant Cell Rep., 8: 521-524.
10. Handique, P.J. and P. Bora, 1999. *In vitro* regeneration of a medicinal plant *Houtuynia cordata* Thunb. From Nodal Explants. Cur. Sci., 76: 1245-1247.
11. Sivanesan, I. and B.R. Jeong, 2007. Direct shoot regeneration from nodal explants of *Sida cordifolia* Linn. In Vitro Cell. Dev. Biol. Plant, 43: 436-441.
12. Balakrishnan, V., R.M. Latha, K.C. Ravinder and P.J. Robinson, 2009. Clonal propagation of *Morus alba* L. through nodal and axillary bud explants. Botany Research International, 2: 42-49.
13. Arora, K., M. Sharma, J. Srivastava, S.A. Ranade and A.K. Sharma, 2010. Rapid *in vitro* cloning of a 40-year-old tree of *Azadirachta indica* A. Juss. (Neem) employing nodal stem segments. Agroforest Syst, 78: 53-63.
14. Tejavathi, D.H. and K. Gayathramma, 1999. Micropropagation through leaf cultures of *Agave vera-cruz* Mill. Int. J. Plant Sci., 2: 113-116.
15. Ignacimuthu, S. and G. Franklin, 1999. Regeneration of plantlets from cotyledon and embryonal axis explants of *Vigna munga* L. Plant Cell Tissue Organ Cult, 55: 75-78.