Influence of PGRS for the *in vitro* Plant Regeneration and Flowering in *Portulaca oleracea* (L.): A Medicinal and Ornamental Plant

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**Abstract:** *in vitro* flowering system was developed to conquer the setback associated with flower development in *in vivo* condition. For this, an efficient, reliable and quick regeneration protocol has been developed for *Portulaca oleracea* L. using nodes of ~1.0-1.5 cm size. Each node procured from young, healthy shoots from garden raised plants. Multiple shoot formation (5.13 ± 0.34) was recorded within two weeks on MS nutrient medium fortified with kinetin (0.5 mg L⁻¹). Shoots were multiplied by subculturing on the same medium after 21 days. On first subculture, *in vitro* flower buds developed, when the developed shoots were maintained for another two weeks on the same medium along with gibberellic acid (0.2 mg L⁻¹). When these flower buds containing shoots were transferred to MS medium containing Indole-3-butyric acid (0.25 mg L⁻¹), *in vitro* roots emerged and the flower buds underwent anthesis. The rooted plantlets were hardened and successfully established in earthen pots with 70% success rate. The present research illustrates *in vitro* flowering system to get rid off problems associated with flower growth and development.

**Key words:** *Portulaca oleracea* L., multiple shoot, micropropagation, flower bud, *in vitro* flowering

**INTRODUCTION**

*Portulaca oleracea* L. (purslane) is a common, herbaceous annual succulent weed and ornamental plant of medicinal value belongs to the family Portulacaceae, which is distributed extensively in temperate and tropical regions of the world (Radhakrishnan et al., 2001). It has been used as a kind of food and medicinal plant for thousands of years in China. *P. oleracea* L. is of abundant nutrition with contents of proteins, carbohydrates, Ca, K, Zn and Na (Abercomand, 2009). As a kind of Chinese traditional medicine, *P. oleracea* L. is very important because of its special medical functions. Traditionally, it has been in use for the treatment of dysentery with bloody stools and externally for boils and sores, eczema, cryspinlas and insect and snake bites (Zhang et al., 2002; Chen et al., 2003; Palaniswamy et al., 2004; Yazici et al., 2007). It is also known to reduce the incidence of cancer and heart diseases (Simopoulos, 1991). It has also been used anti-septic, anti-scrotic, anti-spasmocidal, diuretic and vermifuge in oral ulcers and urinary disorders (Leung and Foster, 1996). At the same time, it has been reported to have anti-bacterial, analgesic, anti-inflammatory, anti-diabetic, anti-hypoxic, anti-oxidant and wound healing properties (Zhang et al., 2002; Xiang et al., 2005; Meng and Wu, 2008; Li et al., 2009; Chen-Jie et al., 2009). These all therapeutic values are attributed by the presence on many biologically active compounds. Some of the biologically active compounds include free oxalic acids, alkaloids, omega-3 fatty acids, coumarins, flavonoids, cardiac glycosides and anthraquinone glycosides. It has high contents of omega-3 fatty acids and protein (Ezekwe et al., 1999).

*In vitro* flowering is an important phase in growth, development and physiological science. It is a difficult phenomenon, is sensitive to the environment. The conversion from vegetative to reproductive growth *in vitro* is thought to be regulated by external and internal factors, which include plant growth regulators, nutrients, the pH of the medium and light conditions (Heylen and Venkrid, 1988). All factors interact in various complex and erratic ways (Tran Thanh Van, 1973, Teixeira da Silva and Nhut, 2003).

Some reports are available on the stimulatory effects of cytokinin on *in vitro* floral morphogenesis in various plant species viz., *Bambusa arundinacea* (Joshi and Nadgauda, 1997), *Cymbidium ensifolium* (Chang and Chang, 2003), *Zantedeschia* sp. (Naore et al., 2004). The mechanism that regulates the transition in plants from...
the vegetative state to reproductive development is poorly understood because the flowering physiology is difficult to study using traditional methods. *In vitro* flowering can reduce the influence of environmental factors and meanwhile, allow for precise control of environment factors and the application of PGRs. It may also offer a means for studying the mechanism of flowering. Furthermore, *in vitro* flowering may be used for rapid breeding of distant varieties by synchronization of flowering.

In the present research, we report an optimal regeneration protocol and new findings on *in vitro* flowering in *Portulaca oleracea* L.

**MATERIALS AND METHODS**

**Collection of plant material and their sterilization:** Healthy nodal segments of *Portulaca oleracea* L. with axillary buds were collected from 5-6 weeks old plants in the month of August, 2008. The plants were growing in earthen pots, maintained for the procurement of experimental material to carry out research work at the Department of Botany, University of Rajasthan, Jaipur (Rajasthan). Nodes were cut into small segments of 1 cm long and thoroughly washed under running tap water for about 15 min. Explants were disinfected with 1% solution (v/v) of teepol for 5 min followed by rinsing with sterile distill water thrice, subsequently surface sterilization of explants using 0.1% HgCl₂ in laminar air flow cabinet and rinsed thrice in sterile distilled water to remove traces of HgCl₂.

**Culture medium and conditions:** Surface sterilized explants were inoculated on semi solid MS medium containing various concentrations and combinations of auxins and cytokinins. Sucrose (3%) was used as the sole carbon source. The pH was adjusted to 5.8±0.2 with 0.1 N NaOH or 0.1 N HCl before autoclaving. The cultures were maintained at 25±2°C under 16 h light/8 h dark cycle at the light intensity of 40 μmol m⁻² sec⁻¹ provided by cool and white fluorescent tubes.

**Shoot multiplication and root induction:** Nodal segments were inoculated on MS medium containing cytokinin BAP and Kn (0.1-5.0 mg L⁻¹) each separately as well as in combination for sprouting the shoots. Shoots sprouted after two weeks of inoculation, these shoots were excised and subculutured on MS medium supplemented with Kn (0.5 mg L⁻¹) each for their further elongation and development. Flower buds emerged on the axil of shoots at terminal portion on the same medium when kept for another two weeks. Subculture of the shoots with flower bud was performed after 21 days on MS medium fortified with auxin (IBA, IAA, NAA and 2, 4-D; 0.1-5.0 mg L⁻¹ each separately) for *in vitro* root induction.

**Effect of cytokinins on floral stimulation:** *In vitro* flowering is positively affected by the presence of cytokinin, which is also apparent in the present study. The role of cytokinin in morphogenesis, especially floral morphogenesis was proved positively during the research work. Cytokinins are constituents of the floral stimulant transported from phloem sap to the apical part stimulating *in vitro* flowering (Bernier et al., 1993). Cytokinins are required to keep up the cell division cycle but might also be involved in promoting the transition from undifferentiated stem cells to differentiation (Werner et al., 2001).

**Hardening and acclimatization of plantlets:** Complete plantlets were taken out from the culture vials (Culture tubes/Flasks) and washed their roots delicately with sterilized distilled water to remove adhered particles of agar. Afterwards, these plantlets were transferred to earthen pots having vermicompost and sterilized, autoclaved soil in the ratio of 3:1 and kept in Green House (Saveer Biotech., New Delhi) at BISR, Jaipur for their hardening, where controlled temperature (28±2°C) and high humidity (80±5%) were maintained for about 3-4 weeks. These hardened plantlets were gradually acclimatized to the natural environment by keeping out these pots having juvenile hardened plantlets to shaded areas, then the timing to the exposure of these plantlets to sunlight subsequently increased from 15 min to 2 h. When these plantlets survived well in the full sunlight, they were finally transplanted to the garden soil.

**Statistical analysis:** Each treatment was repeated at least three times and each treatment having 8 replicates. Data was recorded periodically. Data was analyzed for significance by analysis of variance with the mean separation by Duncan’s multiple range test (Duncan, 1955).

**RESULTS AND DISCUSSION**

**Multiple shoot induction:** During the present research endeavor, the nodal segments of *Portulaca oleracea* L. (1.0-1.5 cm) inoculated on MS medium supplemented with different concentrations of Kn induced varied number of shoots. The number of shoots produced per explants increased with an increasing concentration of Kn up to 0.5 mg L⁻¹, was found to be optimum for inducing
Fig. 1: A-E in vitro plant regeneration and flowering in *Portulaca* (L.): a-b: proliferation of axillary buds on MS medium containing Kn (0.5 mg L\(^{-1}\)) after two weeks of inoculation; c: multiplication and elongation of shoots on the same medium after subculturing; d: *In vitro* root introduction on MS+IBA (0.25 mg L\(^{-1}\)) along with flowering in the regenerated plantlet on the MS medium fortified with Kn (0.5 mg L\(^{-1}\)) and GA\(_3\) (0.2 mg L\(^{-1}\)) after another two weeks and e: *In vitro* rooting on Ms medium containing IBA (0.25 mg L\(^{-1}\)).

Table 1: Effect of cytokinins in MS basal medium on shoot proliferation from nodal segments of *Portulaca oleracea*

<table>
<thead>
<tr>
<th>Cytokinin (mg L(^{-1}))</th>
<th>% explants showing shoot regeneration</th>
<th>Average No. of shoots/explants <em>Mean±S.E.</em></th>
<th>Average length of shoot (cm) <em>Mean±S.E.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Kn (0.1)</td>
<td>35</td>
<td>2.11±0.3</td>
<td>2.16±0.3</td>
</tr>
<tr>
<td>Kn (0.2)</td>
<td>45</td>
<td>3.21±0.4</td>
<td>4.13±0.4</td>
</tr>
<tr>
<td>Kn (0.3)</td>
<td>48</td>
<td>4.15±0.4</td>
<td>6.25±0.2</td>
</tr>
<tr>
<td>Kn (0.4)</td>
<td>66</td>
<td>5.13±0.3</td>
<td>7.23±0.3</td>
</tr>
<tr>
<td>Kn (1.0)</td>
<td>75</td>
<td>3.18±0.3</td>
<td>5.15±0.2</td>
</tr>
<tr>
<td>BAP (0.1)</td>
<td>15</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>BAP (0.2)</td>
<td>25</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>BAP (0.3)</td>
<td>33</td>
<td>2.14±0.3</td>
<td>4.12±0.2</td>
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<tr>
<td>BAP (0.4)</td>
<td>45</td>
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<td>2.14±0.3</td>
</tr>
<tr>
<td>BAP (1.0)</td>
<td>68</td>
<td>NIL</td>
<td>NIL</td>
</tr>
</tbody>
</table>

*The frequency of different stages of somatic embryos was determined by taking the samples from eight replicates per 2. 4-D treatment. Embryo percentage denotes the number of different embryos to the total number of embryos formed per flask.

maximum number of shoots (5±0.2) with highest response of shoot regeneration (86%) (Fig. 1 A-C; Table 1). The efficiency of shoot elongation could be increased on to the same medium, when these shoots were left for another one week. These results are in consonance to the findings reported by Walia *et al.* (2007). In contrary to the present findings, BAP proved to be better to stimulate multiple shoot induction in *Quercus euboica* (Kartsonas and Papafothou, 2007), *Eclipta alba* (Dhaka and Kothari, 2005), *Hibiscus sabdariffa* L. (Gomez-Leyva *et al.*, 2008) and *Perilla frutescens* L. Britton (Hossain *et al.*, 2010). Further, BAP in combination with TDZ and tyrosine revealed efficient responses in order to stimulate multiple shoots in *Artemisia vulgaris* L. (Kumar and Ranjitha, 2010).

**In vitro flowering:** Cytokinins are essential for *In vitro* flowering. At the same time, they regulate cell division and organogenesis (Scorzà, 1982). *In vitro* flower bud induction was observed when these shoots were kept for another two weeks on the same shoot multiplication medium along with GA\(_3\) (0.2 mg L\(^{-1}\)) (Fig. 1D). In consonance to the present results, the presence of GA\(_3\) is important for the induction of flower buds as reported by
Stephen and Jayabalai (1998) in Coriandrum sativum L. and Thiruvengadam and Jayabalai (2001) in Vitex negundo L. In contrary to the present findings, the flower buds developed on the BAP supplemented MS medium in a diverse plant species e.g., ginseng (Chang and Hsing, 1980), Phalanopsis (Duan and Yazawa, 1995), Calamus thwaitesi Becc. (Ramanayake, 1999) and Murraya paniculata (Jumin and Ahmad, 1999).

In vitro rooting: Flower buds containing shoots were rooted on MS medium along with IBA (0.25 mg L⁻¹) after 7 days of incubation in the plant growth chamber (Fig. 1e). Sen et al. (2009) reported in vitro rooting in Phyllanthus amarus Schum. and Thonn. on MS medium fortified with IBA is in consonance to the present findings. In contrast to the present study Zhang et al. (2005) reported in vitro rooting in Perilla frutescens L. Britton in the absence of external hormones. At the same time, Min Kim et al. (2010) also reported optimum in vitro root induction in MS medium along with NAA in Cucurbita ficifolia Bouche. Further, BAP (0.3 mg L⁻¹) and activated charcoal (0.2%) was found to be effective for the stimulation of in vitro root induction in Citrullus colocynthis (Satyavani et al., 2011).

After the proper root development, the shoot underwent anthesis. Later on, complete flower was reported in in vitro condition. Whenever, the plantlets developed in vitro, these plantlets were removed from the in vitro condition and transferred to the earthen pots for further hardening in the plant growth chamber. The plantlets were hardened by the procedure mentioned earlier. The same procedure for hardening the tissue culture raised plants of Bacopa monnieri was adopted by Soundaranjan and Karunanidhi (2011). In contrary, Deb and Imchen (2010) acclimatized and hardened the tissue culture raised orchid seedlings in a different way in which they used 1/10th liquid MS salts, tap water with charcoal chips, bricks and decayed wood as an alternative substratum.

CONCLUSION

The present study illustrates the role of cytokinin in the development of in vitro plant regeneration and flowering. In vitro flowering also has practical connotation for the possible improvement in the reproductive efficiency of Portulaca oleracea by hormonal manipulation. Moreover, the results might increase our understanding of the flowering process in purslane and could help micropropagators for in vitro flower induction.

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level of lipid peroxidation and proline accumulation.
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regeneration in vitro directly from cotyledon and
hypocotyls explants of Perilla frutescens and their