High Frequency Invitro Regeneration In Bryonopsis Laciniosa(L.)Naud An Endangered Medicinal Plant

*V. J. E. Caroline

Biotechnology Laboratory, Dept. of Botany, Kakathiya University, Warangal-506009, A. P., India
*Corresponding Author E-mail: carolynvaikuntam@gmail.com, vjecaroline@yahoo.com

Abstract

*Bryonopsis laciniosa(L.)Naud* is an endangered medicinal plant commonly known as lollipop climber belongs to the family Cucurbitaceae. The plant is bitter and aperient and is considered to possess tonic properties. *Bryonopsis* is used in folk, unani and sidha. The aim of the present study was to develop an efficient protocol for high frequency *invitro* regeneration. Regeneration was observed from nodal, leaf, cotyledon and stem explant. High frequency regeneration was achieved from nodal explants on MS basal medium fortified with 2.0 mg/l BAP and 0.5 mg/l TDZ. *Invitro* grown shoots were cultured for rooting on half strength MS medium supplemented with 1.0 mg/l IBA. The rooted plantlets were transferred to greenhouse and maintained for one to two weeks and later on transferred to manure soil with 90% survival.

**Keywords:** Folk, Unani, Sidha, *Bryonopsis laciniosa*, lollipop climber

Introduction

*Invitro* plant tissue culture technique is an excellent tool for mass multiplication and conservation of endangered and threatened medicinal plants (Anis and Faisal, 2005). *Bryonopsis laciniosa(L.)Naud* is an endangered and medicinally important plant commonly known as lollipop climber belongs to the family Cucurbitaceae. The plant is bitter and aperients and is considered to possess tonic properties. Various parts of the plant acts as insect repellent, laxative, anodyne and is used in Folk, Unani and Sidha.

The bioactive molecules triterpene glycosides, saponin and goniotohalamin were isolated from the whole plant. Goniotohalamin a bioactive compound isolated from the whole plant showed potent cytotoxicity, weak antibacterial and significant antifungal activity against a wide range of gram negative bacteria and fungi (Mosaddik, M. A and
M. Ekramul Haque, 2003). Cytotoxic activity of(s) goniothalamin and analogues isolated from the medicinal plant *Bryonopsis* were evaluated against eight human cancer cells(Fatima *et al.*, 2006). Organogenesis refers to the induction of morphologically well defined organs like shoots, roots etc. from callus cultures. Plant regeneration through adventitious bud formation can either be directly from the initial explants or through a callus phase.

Regeneration from explants of *Cucumis sativus* such as cotyledon, hypocotyl and leaf through callus phase or direct regeneration (Ziv and Gadasi, 1986; Malepszy, 198; Sang *et al.*, 1988; Gambley and Dodd, 1990). Plantlet formation has already been reported in *Cucurbit pepo* (Jelaska, 1974, Rahman *et al.*, 1991:1993). Watermelon (Dong and Jia, 1991), Cucumber(Chee, 1990). Teasle gourd(Nabi *et al.*, 2002). Different parts of plant cells, tissues and organs could be cultured successfully to regenerate into whole plants (Tisserat and Murashige, 1977; Narayana swamy, 1977; Vasil 1980; Evans *et al.*, 1983, Amirato, 1983). The degree of regeneration varies considerably from species to species(Tisserat 1987).

The frequency of regeneration in the earlier studies was depend on the source of explant, cultivar, and growth regulator combinations. Frequency maturation of embryos and regeneration of plantlets was achieved only after repeated sub-culture, previous reports on members of Cucurbitaceae were focused on establishing procedures for regeneration of different cultivars of *Cucumis*(Punja *et al.*, 1990; Chee, 1991). There has been progress in tissue culture studies in many Cucurbitaceae members such as *Momordica dioica* (Shiragave and Chavan, 2001), *Coccina indica* (Venkateswarlu *et al.*, 2001). *Citrullus vulgaris*(Dong & Jia, 1991), *Cucumis melo*(Mackay *et al.*, 1989) and *Coccinia grandis*(Anugulati, 1988). But no such invitro culture studies have been carried out in this valuable medicinal climber, and there were no studies on regeneration of *Bryonopsis laciniosa*, so adequate information on this aspect is not available.

The main objective of this study was to establish an efficient protocol for high frequency invitro plant regeneration.

**Materials and Methods**

Seeds and plants were collected from the local forest area of Warangal dist. and developed in the campus garden. The seeds of *Bryonopsis laciniosa*(L. ) *Naud* were surface sterilized with 0. 1% Hgcl$_2$ for 5-6 minutes and rinsed in serile distilled water thrice. The seeds were allowed to germinate on paper bridge. Cotyledon, stem, nodal and leaf explants were excised from seedlings. The explants were cultured on MS basal medium supplemented with different concentrations of plant growth regulators along with 3% sucrose(w/v) and 0. 8%(w/v) of agar. The PH of the medium was adjusted to 5. 7 to 5. 8 using 0. 1N NaOH or 0. 1NHCl before autoclaving. About 10ml of the medium were dispensed in each culture tube and sealed with non absorbant cotton plugs prior to autoclaving. The culture tubes were autoclaved at 121°C for 20 minutes and maintained at 16hr photoperiod with 2000 lux light.
Acclimatization of the plantlets
The *invitro* regenerated plantlets were taken out from rooting medium and washed gently to remove the culture media and planted in plastic pots containing soil-rite. The plantlets with pots covered with polythene bags to check excessive transpiration are transferred to green house and maintained for one to two weeks and later on, the polythene bags were removed and transferred to manure soil for hardening.

Results and Discussions
In the present study BAP, IBA and TDZ in combination proved to be suitable for morphogenesis and regeneration.

Nodal explants was inoculated on MS basal medium supplemented with 1.0 mg/l BAP and 0.5 mg/l IBA initiated callus(plate1, fig1). A friable callus was induced after two weeks of sub-culture on the same composition of medium (plate1, fig2). Single shoot was induced from callus by increasing the concentration of IBA(1.0 mg/l) on MS medium fortified with 1.0mg/l BAP (Plate1, fig3) IBA played an important role in the formation of organogenesis. High frequency regeneration was achieved on MS medium fortified with 2.0 mg/l BAP and 0.5mg/l TDZ(plate1, fig4). The regenerated micro shoots were sub-cultured on the same composition of medium for further shoot proliferation and elongation.

Plate 1: Induction of callus, rhizogenesis and regeneration from nodal explant cultures of *Bryonopsis laciniosa* (L.) Naud

Fig 1: Initiation of callus from nodal explant on MS + 1.0 mg/l BAP + 0.5 mg/l IBA. Fig 2: Induction of friable callus on the same medium after two weeks of sub-culture. Fig 3: Induction of single root directly from the callus on MS + 1.0 mg/l BAP + 1.0 mg/l IBA. Fig 4: Regeneration of callus derived from nodal explants on MS+2.0 mg/l BAP + 0.5 mg/l TDZ.

In the present study, BAP and TDZ showed efficient response and number of shoots varied with different concentrations and combinations of BAP and TDZ, out of the combinations tested 2.0 mg/l BAP + 0.5 mg/l TDZ showed the highest response.
in nodal explants with maximum number of shoots (12), shoot elongation (2.0) and the percentage of shoot development (80.8) after three weeks of inoculation (table 1). The frequency of shoot regeneration was declined at higher concentrations of growth regulators. Similar response was observed in *Cajanus cajan* (Singh et al., 2002).

MS medium supplemented with 2.0 mg/l Kn and 1.0 mg/l L-glutamic acid by adding 20% CM observed plantlet formation from leaf derived callus (plate 2, fig 1). Cotyledon explants derived calli when cultured on MS medium fortified with 2.0 mg/l Kn and 0.5 mg/l TDZ and 20% CM induced regeneration and produced a single shoot (plate 2, fig 2). MS medium supplemented with 2.0 mg/l TDZ +1.0 mg/l IBA +1.0 mg/l L-Glutamic acid and 20% CM induced rhizogenesis and shoots from stem derived callus (plate 2, fig 3).

The percentage of culture response in inducing callus and regeneration from callus derived from nodal is high when compared to leaf, cotyledon and stem.

*In vitro* grown healthy micro shoots were cultured on half strength MS medium supplemented with different concentrations of IBA (3.0, 2.5, 2.0, 1.5, 1.0 and 0.5 mg/l). Adventitious roots were developed from the base of the shoots after two weeks of culture on rooting medium. Maximum number of roots were developed in the presence of 1.0 mg/l IBA (plate 2, fig 4).

**Plate 2:** Induction of rhizogenesis and regeneration from leaf, cotyledon, stem and nodal explant cultures of *Bryonopsis laciniosa* (L.) Naud

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**Fig 1:** Regeneration of plantlets (two) on MS + 2.0 mg/l Kn +1.0 mg/l L-glutamic acid + 20% CM. **Fig 2:** Induction of regeneration (a single shoot) from cotyledon derived callus on MS +2.0 mg/l Kn +0.5 mg/l TDZ + 20% CM. **Fig 3:** Rhizogenesis and shoot induction from stem derived callus on MS +2.0 mg/l TDZ +1.0 mg/l IBA +1.0 mg/l L-Glutamic acid + 20% CM. **Fig 4:** Induction of maximum number of roots from nodal derived callus on MS + 1.0 mg/l IBA.

The rooted plantlets were transferred to natural environment after proper acclimatization. The acclimatized plantlets successfully showed 90% survival after hardening.
In the present investigation, it was found that the balanced cytokinins and auxins are necessary for the perfect regeneration. High concentration of auxin to cytokinin promotes roots whereas high concentration of cytokinin and low auxin favors shoot bud formation (Lang and Kohlenbach, 1979).

In the present investigation, the BAP and TDZ induced shoot buds and Kn in combination of L-glutamic acid were also proved to develop plantlets. BAP or TDZ proved as the most effective cytokinin than Kn in inducing shoot formation in Capsicum (Phillips and Hustenberger, 1985; Sripichit et al., 1987). Gunay and Rao (1978) was reported that no shoot bud formation was observed in red pepper when Kn was used. NAA in combination with either BAP or Kn has shown stimulatory effect on shoot bud formation in Brassica (Wong and Loh, 1987). George and Rao (1980) reported shoot buds on medium containing NAA and BAP.

Regeneration of plantlets were made on MS supplemented with different concentrations of Kinetin(1.0 mg/l-3.0 mg/l) in combination with aminoacids(L-glutamic acid). Aminoacids played major role in induction of shoots, Anitha and Pulliaah (2002) in Decalepis hamiltonii and Latha et al., (1988) in Porteressia coarctata also demonstrated similar results.

The BAP is the most efficient cytokinin in promoting adventitious shoot formation in many plants (Pirek, 1987). The stimulatory effect of BAP was tested for multiple shoot regeneration in several medicinal and aromatic plants (Pattnaik and Chand, 1996; Saxena et al., 1997; Khalefalla and Hattori, 1999; Begum et al., 2000, Faisal et al., 2006). It was demonstrated that, the different concentrations of BAP and IBA combinations and BAP and TDZ combinations were suitable hormones for induction of shoot and root from nodal explants of Bryonopsis (Table 2). This is in accordance with the results as reported earlier (Kulkarni et al., 2002; Yokoya and Handro 2002; Nishikoshta and Bansal 2002; John Britto et al., 2001; Reddy et al., 1998; Deora and Shekhawat 1995).

IBA is found as the most potential auxin in inducing roots from the regenerated shoots (Martin, 2002; Chandramu et al., 2003).

**Table 1:** Effect of cytokinin for shoot regeneration from nodal explants of *Bryonopsis laciniosa* (L.) Naud

<table>
<thead>
<tr>
<th>Hormone conc (mg/l)</th>
<th>No. of explants cultured</th>
<th>Callus regeneration</th>
<th>No. of shoots</th>
<th>Shoot length</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP+TDZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5+1.0</td>
<td>25</td>
<td>15.4</td>
<td>2.0</td>
<td>0.7</td>
</tr>
<tr>
<td>1.0+1.0</td>
<td>22</td>
<td>28.0</td>
<td>4.5</td>
<td>1.1</td>
</tr>
<tr>
<td>1.5+0.5</td>
<td>21</td>
<td>50.1</td>
<td>8.1</td>
<td>1.5</td>
</tr>
<tr>
<td>2.0+0.5</td>
<td>24</td>
<td>80.8</td>
<td>12</td>
<td>2.0</td>
</tr>
<tr>
<td>2.5+0.5</td>
<td>23</td>
<td>41.2</td>
<td>6.3</td>
<td>1.4</td>
</tr>
<tr>
<td>3.0+1.0</td>
<td>28</td>
<td>32.3</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>4.0+1.0</td>
<td>26</td>
<td>20.5</td>
<td>1.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Table II: Effect of combination of BAP+IBA and BAP+TDZ on morphogenetic response from nodal explant of Bryonopsis laciniosa (L.) Naud.

<table>
<thead>
<tr>
<th>Hormones</th>
<th>% of callus with Growth response</th>
<th>Morphogenetic response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 BAP+0.5 IBA</td>
<td>16</td>
<td>Normal callus growth</td>
</tr>
<tr>
<td>1.0 BAP+0.5 IBA</td>
<td>20</td>
<td>Compact callus</td>
</tr>
<tr>
<td>1.5 BAP+0.5 IBA</td>
<td>28</td>
<td>Initiation of callus</td>
</tr>
<tr>
<td>2 BAP+0.5 IBA</td>
<td>26</td>
<td>Slow callus growth</td>
</tr>
<tr>
<td>1.0 BAP+1.0 IBA</td>
<td>34</td>
<td>Single root formation</td>
</tr>
<tr>
<td>1.0 BAP+2.0 IBA</td>
<td>30</td>
<td>Green spot on callus</td>
</tr>
<tr>
<td>1.0 BAP+0.5 TDZ</td>
<td>26</td>
<td>Friable callus</td>
</tr>
<tr>
<td>1.5 BAP+0.5 TDZ</td>
<td>24</td>
<td>Globular callus</td>
</tr>
<tr>
<td>2.0 BAP+0.5TDZ</td>
<td>35</td>
<td>Regeneration</td>
</tr>
<tr>
<td>2.5 BAP+0.5 IBA</td>
<td>21</td>
<td>Compact callus</td>
</tr>
<tr>
<td>3.0 BAP+0.5 TDZ</td>
<td>18</td>
<td>Soft callus</td>
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</tbody>
</table>

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High

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