In vitro Study on Regeneration of Gladiolus grandiflorus Corm Calli as Affected by Plant Growth Regulators

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Abstract: In this study, in vitro organogenesis of Gladiolus grandiflorus cultivar pink corn segments were evaluated by culturing corn calli in modified MS medium supplemented with 3% sucrose and 0.7% agar with different concentration of BAP (0, 1, 2 and 4 mg L\(^{-1}\) medium) and NAA (0, 0.5, 1 and 2 mg L\(^{-1}\) medium) in factorial experiment of Completely Randomized Design (CRD). In order to obtain Gladiolus calli, corn segments (approx. 5×5×1 mm in size) were kept in modified MS medium (Murashige and Skoog, 1962) that was supplemented with 1 mg L\(^{-1}\) 2, 4-D, 3% sucrose and 0.7% agar. The results showed that increasing the concentration of BAP from 0 to 2 mg L\(^{-1}\) medium simulated plantlet regeneration but no significantly effect was obtained on shoot and corncob organogenesis between 2 and 4 mg L\(^{-1}\) BAP concentration in medium. Increasing of NAA content in media without BAP developed rootlet significantly. Interaction results showed that increasing BAP content against decreasing of NAA concentration stimulates the shoot and corncob proliferation.

Key words: BAP, Gladiolus grandiflorus, MS medium, NAA, organogenesis

INTRODUCTION

Gladiolus, an important bulbous ornamental plant, is one of the most important and popular herbaceous cut flowers with remarkable commercial value in Iran as well as in other countries. It has long lasting flower stalks, attractive colors and numerous forms that these make it an all-time favorite for the flower lovers and is said to be the queen of bulbous flowers (Ahmad et al., 2000; Nagaraju et al., 2002).

The species and varieties of the genus Gladiolus are varying because it includes 180 species with more than 10,000 cultivars (Sinha and Roy, 2002; Roy et al., 2006). However about 20 of them are grown for commercial purposes (Sinha and Roy, 2002). Annually cultivated gladioli are propagated vegetatively by corms and cornets. The cornet is auxiliary bud on the corm which is a compressed thickened stem and as the resting perpetuating organ (Steinitz et al., 1991; Remotti and Loffler, 1995; Sen and Sen, 1995; Ahmad et al., 2000; Nagaraju et al., 2002; Sinha and Roy, 2002). This kind of propagation is time consuming because most cultivars have a very slow rate of multiplication, also disease factors such as fungi, bacteria and viruses that exist in corms usually result in decrease of growth and flowering in this plant (Darvishi et al., 2006). Therefore there are highly desirable exist to develop methods to increase rate of multiplication as well as disease-free stocks (Singh et al., 2007). During the last two decades, biotechnologies have been adapted to agricultural practices and have opened vistas for plant utilization. Tissue culture technique is one of the most important components of plant biotechnology and being widely used for producing of high quality large-scaled plant multiplication, can play a major role to achieve a large number of planting material with genetically identical, physiological uniform and developmentally normal plants with the ability to survive upon transfer to ex vitro conditions in a relatively short period of time with the added advantage of maintaining disease-free stock.

It is demonstrated that callus regeneration mainly depends on cultivated varieties, explants and growth regulators used in culture media (Sinha and Roy, 2002). Organogenesis refers to de novo differentiation of callus or other tissues into adventitious meristems that can form stems, leaves, roots and cornets and ultimately plants (Frey and Jarvik, 1991; Zaidi et al., 2000). Growth regulator concentrations such as auxin and cytokinin
have been added as promoting factors in the culture medium, are critical to the plant regeneration and control of growth and morphogenesis (Zaidi et al., 2000).

In vitro propagation has been developed to accelerate the multiplication efficiency. In this regards, growing and multiplication of cells, tissues and organs on defined solid or liquid media can play a major role with the added advantages of maintaining clean stocks (Frey and Junick, 1991). Recently there are many attempts to in vitro propagation of Gladiolus (Sen and Sen, 1995; Dantu and Bhojwani, 1995; Kumar et al., 1999; Ahmad et al., 2000; Zaidi et al., 2000; Prasad and Gupta, 2006; Roy et al., 2006) under aseptic and controlled conditions developing a small cornets at the base of shoot (Ahmad et al., 2000; Darvishi et al., 2006).

The presenting study has been undertaken to examine the effect of different concentrations of 6-benzylaminopurine (BAP) and Naphthalene Acetic Acid (NAA) in modified MS medium supplemented with sucrose, on organogenesis of Gladiolus grandiflorus cv. pink corn calli.

**MATERIALS AND METHODS**

Experiment was carried out at tissue culture laboratory of the Junior College of Agriculture, Mohaghegh Ardabili University, Ardabil, Iran, at 2007. Healthy Gladiolus grandiflorus cornets cv. pink (10-20 mm in diameters) at the end of dormancy were used as source of material in this study. The cornets were soaked in water for 24 h and then washed thoroughly under running water for 60 min. The cornets were sterilized by immersion for 15 min in sodium hypochlorite solution (containing; 2.5% w/v NaClO, 0.2% v/v Tween20, H2O) for 10 min and finally the explants were washed in sterile distilled water for 3 times in a laminar air flow cabinet.

For establishment, following surface disinfection and washing, cornets were placed on autoclaved MS basal medium (Murashige and Skoog, 1962) containing 2% sucrose. The pH of the media was adjusted 5.7±0.1 with 0.1 N HCl and NaOH prior to adding with 0.7% (w/v) agar (Difco Bacto) and autoclaving at 121°C for 20 min. Culture bottle were kept in 25±1°C, in growth chamber for 10 days.

For callus induction, established cornets were excised (5×5×1 mm approx.) using a sharp surgical knife in a laminar air flow cabinet and subcultured in 150 mL glass flask containing 25 mL MS basal medium supplemented with 1 mg L⁻¹ 2, 4-D, 3% sucrose and 0.7% agar. The pH of medium was adjusted to 5.7±0.1 before autoclaving for 20 min at 121°C. Cultures were incubated in temperature 25±1°C and relatively humidity of 60% in dark for 4 weeks. To obtain extra calli, they were subcultured in same media frequently. The friable calli were then transferred to regeneration medium.

In order to evaluate organogenesis (Shoot and root, cornel inducing), the MS medium was supplemented with two plant regulators, benzylaminopurine (BAP) and Naphthalene Acetic Acid (NAA) for checking their role and the best concentrations in development organogenesis. Various concentrations of BAP and NAA ranging from 0, 1, 2 and 4 mg L⁻¹ and 0, 0.5, 1 and 2 mg L⁻¹, respectively were prepared in basal MS medium supplemented with 3% sucrose and 0.7% agar. Both individual and combined effects of BAP and NAA were tested. Corm calli (had a mean fresh weight of 6±2 g) cultured in treated medium and were placed at 25±1°C, continuous light (3000 Lux) with a photoperiod of 16 h daily and 60-70% relatively humidity. Their simple and interactive effects were analyzed in factorial experiment (with 2 factor; BAP in 4 levels; 0, 1, 2 and 4 mg L⁻¹ NAA in 4 levels; 0, 0.5, 1 and 2 mg L⁻¹) of Completely Randomized Design (CRD). There were 5 replications per treatment combination and 20 samples per replicate. After three months culturing data were collected through parameters such as production proto cornets-like bodies, number of shoots and number of roots per culture. The means were analyzed by analysis of variance (ANOVA) and Least Significant Difference (LSD) test at 5% significant level with SAS (1985) software (SAS Institute Inc., Cary, NC, USA).

**RESULTS AND DISCUSSION**

The pathways of regeneration, in tissue culture, depend on the media and the material used. Many substances have been added to culture media to enhance plant regeneration. Growth regulators concentrations in the culture medium are critical to the control of growth and morphogenesis. Several workers using various methods and plant parts for regeneration of Gladiolus (Hussey, 1977; Ziv, 1979; Bajaj et al., 1983) as well as Kim et al. (1991) reported the differentiation of shoots, roots and cornets from Gladiolus calli. In this study we evaluated regeneration of Gladiolus grandiflorus cultivar pink corn segments callus. For this, MS medium with 3% sucrose was supplemented with two plant growth regulators, BAP and NAA in order to check their role in the development of the shoot, cornel and root of Gladiolus callus.
Table 1: Effect of 6-benzylaminopurine (BAP) and naphthalene acetic acid (NAA) on shoot regeneration, cornel formation and root formation

<table>
<thead>
<tr>
<th>Treatment combination No.</th>
<th>Treatment combination</th>
<th>Mean root formation per explant</th>
<th>Mean cornel formation per explant</th>
<th>Mean shoot regeneration per explant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>12.2×</td>
<td>0.2f</td>
<td>0.0d</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>14.6f</td>
<td>1.6f</td>
<td>0.4d</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>15.6b</td>
<td>2.0f</td>
<td>0.6d</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>20.8a</td>
<td>1.8f</td>
<td>0.4d</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>1.0g</td>
<td>3.2f</td>
<td>2.6d</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>4.2ef</td>
<td>5.6de</td>
<td>8.6c</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>5.8e</td>
<td>12.0c</td>
<td>16.0b</td>
</tr>
<tr>
<td>8</td>
<td>2.0</td>
<td>8.8d</td>
<td>12.8c</td>
<td>15.6b</td>
</tr>
<tr>
<td>9</td>
<td>0.5</td>
<td>0.0g</td>
<td>9.6d</td>
<td>16.2b</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>0.4g</td>
<td>12.6c</td>
<td>18.0b</td>
</tr>
<tr>
<td>11</td>
<td>2.0</td>
<td>2.6f</td>
<td>18.2a</td>
<td>25.4a</td>
</tr>
<tr>
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<td>0.0</td>
<td>5.8e</td>
<td>19.4a</td>
<td>28.4b</td>
</tr>
<tr>
<td>13</td>
<td>4.0</td>
<td>0.2g</td>
<td>13.6c</td>
<td>19.0b</td>
</tr>
<tr>
<td>14</td>
<td>0.5</td>
<td>0.2g</td>
<td>13.8c</td>
<td>17.8b</td>
</tr>
<tr>
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<td>1.0</td>
<td>0.0g</td>
<td>22.0a</td>
<td>28.0a</td>
</tr>
<tr>
<td>16</td>
<td>2.0</td>
<td>0.0g</td>
<td>18.4a</td>
<td>24.0a</td>
</tr>
<tr>
<td>LSD value (α = 0.05)</td>
<td></td>
<td>3.2</td>
<td>4.4</td>
<td>4.3</td>
</tr>
</tbody>
</table>

*Mean values followed by the same letter(s) are not significantly different at the 5% level (Least Significant Difference (LSD)).

Prasad and Gupta (2006) demonstrated that a combination of NAA and BAP is necessary to induce multiple shoot buds. Also Ziv and Lilen-Kipnis (1990) reported similar requirement of a growth regulator combination of NAA/BAP for in vitro shoot regeneration of *Gladiolus* in medium. However a difference in optimum combination of NAA and BAP was evident with different culture type and media composition. Furthermore the optimum concentration of BAP/NAA required for shoot multiplication varies from cultivar to cultivar (Hussey, 1977). In our work, BAP and NAA at various concentrations were tested for their effects on in vitro organogenesis (Table 1). Callus inducing medium, MS medium with 1 mg L⁻¹ 2, 4-D and 3% sucrose, showed significantly good response in production of extra calli (Fig. 1C). When the corn calli were transferred to the different media supplemented with tested concentrations of BAP and NAA, they started proliferation after 4-5 weeks (Fig. 1D). In media without BAP and NAA no shoot regeneration were observed. This result agrees with that of obtained by Prasad and Gupta (2006) that without combination of NAA and BAP calli have not induced shoot regeneration (Table 1). However in vitro shoot production of *Gladiolus* from corn calli in media with different concentration of BAP can be occurred without NAA (Table 1). Therefore the results confirm that BAP is necessary for shoot induction. Similar trend in shoot formation was reported by De Bruyn and Ferreira (1992). Multiple shoot formation between tested concentrations was observed in combination of 2/1 and 4/2 or 4/1 mg L⁻¹ BAP/NAA concentrations in culture medium. Also our study indicated that BAP in 2 or 4 mg L⁻¹ concentrations in the medium plays same role and increasing BAP concentration from 2 to 4 have no significantly effects on shoot forming. In addition, 2 mg L⁻¹ BAP in combination with 1 mg L⁻¹ NAA were evaluated suitable result as compared to all other media used for shoot and cornel proliferation (Fig. 1D, E, F and G). Kumar et al. (1999) showed that BAP at lower concentrations (<10 mg L⁻¹) promoted shoot differentiation whereas higher concentration (>10 mg L⁻¹) resulted in callus proliferation and inhibited shoot differentiation.

The best cornel formation achieved in culture growing on high BAP and NAA medium. Media containing 2 or 4 mg L⁻¹ BAP injunction with 1 or 2 mg L⁻¹ NAA were high effective in cornel inducing and no significant influence were detected these media.

About root formation, the results indicated that NAA concentration plays an important role in in vitro root multiplication in *Gladiolus*. The best result was obtained on media without BAP (Fig. 1H). It was established that 2 mg L⁻¹ concentration of NAA was optimal for root formation in callus cultures without BAP in MS medium supplemented with 3% sucrose (Table 1).

In addition based on the present study, high BAP and low NAA concentrations in the medium results in the formation of shoot morphogenesis whereas NAA alone or with a very low concentration of BAP is important in the induction of root (Fig. 11).
Fig. 1: (A): Established cornlet segment. (B): Initiation stage of callus. (C): Friable callus developed from established cornlet in MS medium with 1 mg L$^{-1}$ 2, 4-D and 3% sucrose after 8 weeks planting on medium. (D, E, F and G): Shoot and cornlet differentiation on MS medium with 3% sucrose containing BAP (2 mg L$^{-1}$) and NAA (1 mg L$^{-1}$), 6, 8, 10 and 12 weeks after culturing respectively. (H): Root, Cornel and shoot formation in rooting medium with 3% sucrose containing 2 mg L$^{-1}$ NAA without BAP. (I): Clumps of regenerated plantlets after 14 weeks on MS medium with 3% sucrose containing BAP (2 mg L$^{-1}$) and NAA (1 mg L$^{-1}$).
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REFERENCES


