Pak. J. Bot. 33 (special issue) 2001

IN VITRO PROPAGATION OF GLOXINIA (*SINNINGIA SPECIOSA*)

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Abstract

Parts of newly formed leaves of Gloxinia were used as an explant and cultured on Murashiage and Skoog (1962) media containing different concentration of auxin and cytokinins. Shoot was induced from leaf explant on MS medium supplemented with 3 mg/l BAP. Shoot initiation generally started within 70-78 days after the formation of shoot primordia. These newly formed shoots were transferred to MS medium with 2mg/l BAP for further multiplication. Micro cuttings of 2-2.5cm in length were harvested from proliferating cultures and transferred to root induction media containing different doses of NAA and IBA. Plantlets grown in this medium for 4-5 weeks could be successfully established in sterile sandy soil following hardening phase of 20 days and nourishment with Hoagland solution.

Introduction

Ornamental plants are economically important as they contribute substantially to the floriculture industry in number of ways. eg. Ornamental plants form an important source of income for many countries like Nether land and France etc.

Gloxinia (Sinningia speciosa) is one of the popular ornamental plants which have good export potential as a pot flower to many countries of the world. It is a tropical plant which prefers plenty of humidity. The plant needs bright light but no direct sunlight. Gloxinia is generally propagated by seed for commercial purpose and seedlings are raised in masses by this method. But the seed propagation is difficult, being very small seeds mortality is very high and plants grown from seed become very expensive. Moreover one from seed does not have strength to grow another year. It has also been reported that seed propagation results in variability (Gill *et al*, 1994) Moreover this method is very difficult to keep plant true to type and pathogen free (Siddiqui and Iqbal, 1993). Gloxinia may also be propagated from bulbs and by leaf cuttings, but this method is rarely used (Kessler, 1999).

In recent years many exporting firms use tissue culture techniques as a routine procedure to obtain large amount of good quality planting material (Hoque *et al*, 1994). This clonal propagation technique generated a good deal of interest among nursery growers and created a tremendous impact on floriculture industry throughout the world. The propagation of Gloxinia through tissue culture using different organs have been reported by Lertphanichkul *et al* (1999) and Scaramuzzi *et al* (1999). The Gloxinia plant developed through tissue culture remains true to type, inexpensive and disease free.

Present investigation is was undertaken to achieve large scale multiplication and faster development of Gloxinia through tissue culture technique using leaf discs as explants.

Materials and methods

In present study the newly formed leaves were used as explants. Leaf explant of about 2-2 cm in size were collected from pot grown Gloxinia plant obtained from Agri Biotech nursery farms. The fully expanded leaf explant was washed thoroughly with tap water. Surface sterilization was carried out by immersing explant in 15% sodium hypochlorite solution for 15 minutes. Explants were then rinsed three times with autoclaved distilled water. Bleached damaged portion were cut-off after sterilization. The leaf discs were placed on MS basal medium supplemented with 0.5mg/l – 5mg/l of BAP for four weeks and subcultured periodically every 3 weeks for 78 days.For multiplication of induced shoots lower conc. of BAP i.e. 0.5mg/l – 3mg/l were added to MS medium. The higher level. decreased shoot formation. Induction response for multiple shoot formation was determined after 4 weeks.

For root induction, MS basal medium containing different doses of NAA i.e. 0.5mg/l- 2mg/l were used. Three percent sucrose was used in all media. The pH of the media was adjusted to 5.7 and was gelled with 0.3% gelrite. The medium was autoclaved at 121 °C and 15 lb pressure for 15 minutes. Cultures were incubated in standard controlled environment.

Results and Discussions

The method of producing large number of identical clones by *in vitro* culture is being routinely used for a wide range of plant species (Biondi, 1986). In *in vitro* propagation, using segments of bulbs, leaf, inflorescence or stem as an explant are successful (Hussrey, 1978; Peck, 1986). Leaf explants of different size were used for shoot induction and their multiplication. It was observed that the leaf discs of 2x2 cm in size provided best response for shoot induction. Lertphanichkul *et al* (1999) also used the leaf explant of the same size for shoot induction in Gloxinia. When the explants of size more than 2x2 cm were used, they did not respond even after four months and contamination was also high in these cultures, while the explants of size smaller than 2x2 cm died within 10 days after inoculation. Two different medias containing different concentrations of BAP were standardized i.e. shoot induction medium and shoot proliferation medium.

Among various concentration of BAP used optimum shoot induction was observed on MS medium containing 3mg/l of BAP. (fig.1) By decreasing the level of BAP shoot initiation response was gradually decreased and dropped to zero at 0.5mg/l. About 80 of the explants initiated shoots in MS medium containing 3mg/l BAP. By increasing the level of BAP gradual decline in shoot initiation was observed.

For multiplication of induced shoots different concentrations of BAP were used. Shoot multiplication was triggered after transferring the cultures to lower concentration of BAP. Among different concentrations 2mg/l of BAP induced maximum shoots (28 plants produced per culture) (fig 5). But gradual decline in number of shoots was observed when concentration of BAP lower than 2 mg/l was used. At the concentration of 1 mg/l of BAP shoots were not only less in number but also compact and stunted in growth. Roest and Bokelman (1975) obtained similar results when they used 1.0 mg/l BAP in the medium for regeneration of Chrysanthemum. Similarly there was gradual decrease in number of shoots when

concentration of BAP higher than 2 mg/l was used. Lertphanichkul *et al.* (1999) also reported that higher level of BAP causes abnormal shoot formation.

Regenerated shoots produced roots when they were cultured in the rooting medium. Effect of different media composition and additives has been shown in Tab. 3. From the table it is indicated that among different concentration of NAA used only 1 mg/l provided best results. While comparatively less rooting was observed when concentration of NAA was increased or decreased. IBA did not show good results. In contrast Scaramuzzi *et al* (1999) obtained best rooting with IBA. However in present study the results of IBA are not satisfactory either alone or in combination with NAA.

No mortality was noted when the plantlets derived *in vitro* were transplanted onto the potting medium for Green house environment. When all rooted plantlets were hardened in the Green house they were found more healthy and vigorous than normal growing plants. They showed vigorous growth with more multiple shoots after first month. Whereas the normal growing plant manifested normal growth with less number of shoots. Early flowering and profuse branching was noticed in *in vitro* raised clones (fig.7). This study revealed a marked superiority of *in vitro* plants over normal growing plants in term of quality and yields. This plant tissue culture technique can widely be used successfully for the mass scale production of premium quality, vigorous and high yielding planting material to meet the local demand and also to earn valuable foreign exchange by exporting excess flowers to other countries.

Table 1. Effect of different concentration of BAP on shoot induction.

Sr. No	BAP (mg/l)	Explants	Shoot induction (%)
1	MS + 0.5	10	0
2	MS + 1.0	10	30
3	MS + 2.0	10	55
4	MS + 3.0	10	80
5	MS + 4.0	10	70
6	MS + 5.0	10	65

Table 2. Effect of different concentration of BAP on shoot proliferation

Sr. No	BAP (mg/l)	No of cultures Used	Average shoots/culture
1	0.5	5	0
2	1.0	5	8
3	1.5	5	19
4	2.0	5	28
5	2.5	5	22
6	3.0	5	13

Table 3. Effect of different concentration of NAA & IBA on root formation.

Sr No	NAA + IBA (mg/l)	No of cultures Used	Roots (%)
1	0.5 +0	5	55
2	1.0 + 0	5	100
3	1.5 + 0	5	95
4	1.5+ 0.5	5	90
5	0 + 1.0	5	30

Figure 1. Plant differentiation from the leaf disc.

Figure 2: Induction of multiple shoot.

Figure 3. Micro cutting for multiplication.

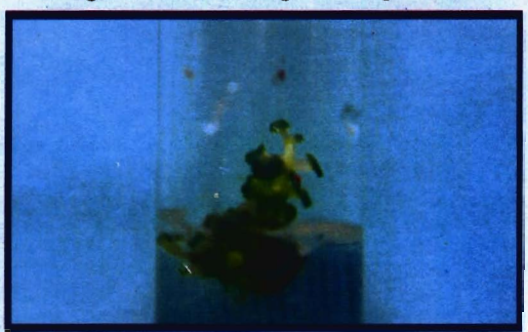

Figure 4. Induction of multiple shoot from micro cutting.

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