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Original Article

IMPROVED PROTOCOL FOR *IN VITRO* PROPAGATION OF GLOXINIA (*SINNINGIA SP.*)

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Abstract: An efficient and reproducible protocol for in vitro multiplication of gloxinia has been developed. Leaf discs used as explants were surface sterilized with $HgCl_2$ (0.1%) and Bavistin (2%). Sterilized explants were cultured on MS media augmented with BAP (2 mg/l) and NAA (0.5 mg/l) which proved most appropriate for shoot induction. Sprouted shoots were sub cultured on medium with different concentrations of BAP which resulted in varying degrees of multiple shoots. Increasing the concentration of BAP resulted in reduction in number of shoots per explant. Maximum proliferation of shoots was observed in MS medium augmented with BAP (2.0 mg/l) and NAA (0.5 mg/l) within 2 weeks and average number of shoots per explant was 7.3. The in vitro raised shoots of gloxinia were successfully rooted in MS media fortified with NAA and IBA. Rooted plantlets could be successfully established in potting mixture of cocopeat and sand (1:1).

Key words: *In vitro, Gloxinia propagation, Sinningia*

INTRODUCTION

Gloxinia (*Sinningia speciosa*) is one of the popular commercial ornamental plant and holds a good export potential as a pot flower in many countries. It belongs to the family Gesneriaceae and is native to Brazil. It is a tropical plant requiring plenty of humidity with no direct sunlight. Gloxinia is a valued plant for beauty and colourful flowers. It is usually propagated by seeds which is quite difficult because of high mortality rates and this makes the plant expensive. Seed propagation also results in varia-bility [1] and the plant is more susceptible to pathogens [2]. Plant can also be traditionally propa-gated by leaf, stem, rhizome and crown cuttings but production of blooming gloxinia take 6-7 months [3].

Micropropagation has been a boon for floriculture as it offers a number of advantages, the most important being the production of enough high quality seedlings to meet demand in short span of time to meet the increasing demand of the planting material. The plants generated through tissue culture remains true-to-type, inexpensive and disease-free. However, before exploiting such a technique for commercial purpose, gathering information regarding requirements for micropropagation and then developing a practicable procedure for best culture condition is a necessity [4]. The present study was undertaken to study various aspects of *in vitro* propagation in order to develop an efficient protocol for mass multiplication of gloxinia.

MATERIALS AND METHODS

Newly formed leaves of gloxinia were collected from the plants maintained in pots and used as explants. The leaves were thoroughly washed under running tap water for half an hour to remove dirt and mud sticking to it. This was followed by continuous shaking with Tween-20 mixed in water for 20 minutes and rinsed with water to remove traces of detergent.

Further aseptic sterilization was carried out under laminar flow chamber using mercuric chloride (0.1%) and Bavistin (2.0%) followed by 3 rinses with autoclaved distilled water. Leaf discs were prepared after cutting off the bleached portions and placed on Murashige and Skoog medium [5] supplemented with different concentration and combination of growth hormones. These were periodically sub cultured after every 3 weeks. The induced shoots were further sub cultured on media with reduced concentration of growth regulators for further multiplication. The shoots obtained on these media were further transferred to the best medium or further proliferation. Individual shoots (4-5 cm) were excised and transferred to rooting media for *in vitro* rooting. All media contained 30 g/l sucrose, 0.1 g/l inositol and 8 g/l agar-agar. The pH of the media was adjusted to 5.8 with 0.1N NaOH and 0.1 HCl before autoclaving at 121°C and 15 psi for 15 minutes. All the cultures were incubated in the culture room at 25$\pm$2°C temperature and 70% relative humidity under 16 hours photoperiod of 1.5 kilolux light intensity provided by cool, white, fluorescent lamps.

The *in vitro* rooted shoots of gloxinia were thoroughly washed with running tap water so as to remove the agar sticking to the roots and were kept in 0.5% Bavistin for about half an hour. These were then transferred to portrays with different combinations of potting mixture viz. cocopeat : sand (1:1), cocopeat: sand: soil (1:1:1), sand: soil: FYM (1:1:1) and cocopeat: soil. These were initially kept covered with jars in order to maintain high relative humidity (80-100%) for 3 weeks. The plantlets were watered 3-4 times a day for a fortnight and afterwards twice a day upto 8 weeks of planting. Observations on the establishment, proliferation, rooting and acclimatization were recorded after 4 weeks of incubation in respective subcultures.

All the experiments were repeated thrice. The data recorded for different parameters were subjected to completely randomized design [6]. The statistical analysis based on mean values per treatment was made using analysis of variance (ANOVA) technique of CRD.

RESULTS

The method of producing a large number of identical clones by *in vitro* culture is being routinely used for a wide range of plant species. A similar attempt has been made to establish an efficient plant regeneration protocol for the rapid multiplication of *Sinningia sp*. The plants were efficiently regenerated from the leaf explants. Leaf discs of different sizes were cut and size of 2x2 cm was most appropriate size responded best for shoot induction.

The standard MS medium augmented with different concentration of growth regulators (BAP and NAA) was used for the shoot induction and multiplication (Table 1). There was no shoot development from the excised leaf explants on the basal medium which indicates growth hormones had a pivotal role in the establishment of cultures. A combination of cytokinin and auxin was used where, BAP (2.0mg/l) and NAA (0.5 mg/l) resulted not only in highest shoot regeneration efficiency (77.7%) but also in maximum number of shoots per explants followed by medium fortified with BAP (3.0 mg/l) and NAA (0.1 mg/l). The *in vitro* raised shoots were further subcultured and maximum multiplication was achieved in the MS medium with BAP (2 mg/l) and NAA (0.5 mg/l) as in induction medium followed by BAP (2.5 mg/l) and NAA (0.5 mg/l). However, it was observed that increase of BAP concentration resulted in decrease in the number of shoots per explant (Table 3).

For *in vitro* induction of roots, individual shoots of 3-4 cm length were transferred to MS medium with IBA and NAA (0.5-2.0 mg/l) individually in presence of 0.1% activated charcoal (Table 3). It was observed that an exogenous auxin supply was necessary to develop proper roots. 100% rooting was observed in MS medium fortified with NAA (1mg./l) in presence of charcoal followed by IBA (2mg/l). However, no root initiation was observed in MS basal medium. Activated charcoal was supplemented in the medium to inhibit the callus formation at the base of the shoot in the rooting medium. The length of the roots was optimum for all concentrations of NAA.

The *in vitro* raised rooted plantlets were acclimatizes by transferring to portrays containing different potting mixtures (Table 4). Maximum survival per cent was achieved in potting mixture cocopeat and sand (1:1) and least survival was observed in potting mixture of sand: soil: FYM (1:1:1).

DISCUSSION

The *in vitro* propagation method standardized in present investigation using leaf discs as explants has

Table 1. Effect of different concentrations of growth regulators in MS medium on *in vitro* establishment and regeneration of cultures of Gloxina. Values in the parenthesis are transformed values.

S. No.	Growth regulators (mg/l)		% of shoot induction	Av. No. of Shoots / explant
	BAP	NAA		
1.	-	-	0 (0.00)	
2.	1.0	0.1	15.43 (23.02)	1.33
3.	2.0	0.1	37.76 (37.88)	2.33
4.	3.0	0.1	71.06 (57.46)	2.66
5.	4.0	0.1	48.86 (44.32)	3.00
6.	1.0	0.5	17.76 (24.83)	2.66
7.	2.0	0.5	77.76 (61.89)	6.00
8.	3.0	0.5	51.06 (45.59)	4.33
9.	4.0	0.5	41.63 (43.04)	2.33
C. D. (0.05)			5.006	

Table 2. Effect of different concentrations of BAP in MS medium on shoot proliferation of Gloxina.

S. No.	BAP (mg/l)	Av. No. of Shoots / explant	Av. length of Shoot (cm)
1.	1.0	3.6	3.7
2.	1.5	6.6	4.4
3.	2.0	12	5.6
4.	2.5	9.3	4.6
5.	3.0	6.3	4.1
6.	3.5	3	3.8

Table 3. Effect of auxin concentrations in MS medium on rooting of *in vitro* raised shoots of Gloxina. Values in the parenthesis are transformed values

S. No	Growth regulators (mg/l)		% of root induction	Av. Length of root (cm)
	NAA	IBA		
1.	-	-	0(0.00)	0
2.	0.5	-	22.2(28.04)	2.2
3.	1.0	-	97.76(84.98)	3.8
4.	1.5	-	82.20(65.10)	3.1
5.	2.0	-	46.63(43.04)	2.5
6.	-	0.5	15.53(23.10)	2.5
7.	-	1.0	51.06(45.59)	3
8.	-	1.5	84.40(66.80)	3.5
9.	-	2.0	94.40(76.42)	3.6
C. D. (0.05)			6.67	

Table 4. Effect of different potting mixtures on hardening of *in vitro* raised rooted plantlets of Gloxinia. Values in the parenthesis are transformed values.

S. No.	Potting mixtures	% survival
1.	Sand: soil: FYM (1:1:1)	15.53(23.10)
2.	Cocopeat	28.83(32.43)
3.	Cocopeat: Sand (1:1)	79.96(63.59)
4.	Cocopeat: Soil (1:1)	35.53(36.55)
C. D. (0.05)		6.29

clearly demonstrated its potential for mass multiplication. Micropropagation can be achieved by means of bulb segments, leaf, inflorescence, shoot tips or stem segments as explants [7,8]. Leaf segments were found to be the best explants [9,10] as these resulted in maximum number of shoots per explants. Naz et al. [11] found 2x2 cm leaf disc to be the most responding size of the explants which is similar to the observation recorded in the present study. Efficient shoot regeneration was achieved from leaf explants in MS medium supplemented with a relatively higher concentration of cytokinin. Shoot development did not occur in absence of exogenous growth hormone. This has also been successfully demonstrated in *Sinningia* by Park et al. [10] where highest shoot regeneration efficiency per explants was attained when MS medium was augmented with BAP (2mg/l) and NAA (0.1mg/l). BAP was shown to have similar influence on shoot induction and regeneration in previous studies on *S. speciosa* [12]. Pang et al. [13] showed that combination of GA_3 and BAP was most effective at inducing the formation of flower buds on sepal segments. Shoot multiplication was rapid and consistent over prolonged periods.

The successful rooting of the *in vitro* raised shoots was achieved on MS medium supplemented with NAA (1mg/l) which is in accordance with the *in vitro* rooting protocols of *Sinnigia sp.* Least rooting (25%) was observed in basal medium which is contradictory to the observations recorded by Park et. al. [10] who obtained 95% rooting after 5 weeks on ½ MS medium without growth regulators. The rooted plantlets were hardened and transferred to the *ex vitro* conditions. A relative humidity of about 85±5% was provided before transferring to greenhouse as was observed by Bharti and Dhiman [14]. The results obtained are useful not only for an efficient regeneration and mass multiplication of *Sinningia* but it may also prove useful as an efficient approach for transformation technique of this valuable plant because of its direct regeneration from leaf explants.

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