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CALLUS INDUCTION, PLANT REGENERATION AND ACCLIMATIZATION OF AFRICAN VIOLET (*SAINTPAULIA IONANTHA*) USING LEAVES AS EXPLANTS

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Abstract

African violet (*Saintpaulia ionantha*) is a very famous ornamental plant due to its various colors and shapes. The present study was conducted to investigate the callus induction and plant regeneration efficiency of African violet and subsequent acclimatization of the regenerated plants. Callus induction efficiency was high with 1.0 mg/L of NAA with MS medium and cotton as a supporting material. Shoot regeneration and multiplication was maximum on full strength MS medium with 3.0 mg/L of BAP and 1.0 mg/L of NAA. Simple MS medium was found effective for the induction of roots in the *in vitro* grown plantlets. Among the different potting mixes used for the acclimatization of rooted plantlets, 100 % sand was found the best. Maximum survival rate was achieved when plantlets were transplanted during winter.

Introduction

Saintpaulia ionantha, commonly known as African violet or *Saintpaulia*, belongs to the family Gesneriaceae. It is a popular commercial and ornamental plant and contains many cultivars with varied colors and shapes. The characteristics that have made *Saintpaulia* such a popular houseplant are visual appeal, tolerance of shaded rooms, ability to flower under artificial light and a remarkable ease of vegetative propagation all the year round (Grout, 1990). African violet is high valued, indoor ornamental plant species. While these plants are propagated most commonly by vegetative leaf cuttings, micropropagation techniques are employed to produce a large number of new and true-to-type plants in a relatively short period of time (Sunpui & Kanchanapoom, 2002). The *In vitro* methods are also widely used for the induction of commercially valuable variability (Mithila *et al*., 2003). To date, micropropagation techniques are very much employed and *In vitro* culture of *Saintpaulia* has been successfully initiated from several sources of explants including leaf (Smith & Norris, 1983), flower bud (Molgaard *et al*., 1991), sub-epidermis (Bilkey & Cocking 1981), anther (Weatherhead *et al*., 1982) and from protoplast (Hoshino *et al*., 1995).

Despite the availability of the sufficient literature on the micropropagation of African violet, there is a need to study the callus induction and plant regeneration efficiency and also provide knowledge regarding the *ex Vitro* requirements of the plantlets i.e., acclimatization.

This paper describes a highly efficient protocol for callus induction, plant regeneration and acclimatization of rooted plantlets of African violet. The presented protocol will certainly provide sufficient knowledge regarding the micropropagation of African violet and also aid in different studies regarding the genetic transformation of *Saintpaulia ionantha*.

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Materials and Methods

Plant material and explant sterilization: The plant material was collected from the nursery of the H.E.J. Research Institute, University of Karachi. Plants were sprayed with the fungicide and insecticide before taking explants. Freshly grown leaves, from the meristematic region, were selected as an explant source. Leaves were washed in running tap water for 10 minutes to remove the dust or sand particles. Then they were surface sterilized by using 0.5% of sodium hypochlorite for 20 minutes. Few drops of Tween-20 were also added as a surfactant. After 20 minutes the plant material was washed three times with sterile distilled water to remove the traces of bleach with gentle shaking under sterile conditions.

Callus induction: The potential of three different PGRs (NAA, IBA and IAA) were analyzed for the induction of callus. Three different concentrations of each PGR (1.0, 2.0 and 3.0 mg/L) were used with MS (Murashige & Skoog, 1962) as a basal medium (Table 1). A simple MS medium (without any PGR) was used as control. All the media formulations had 3% of sugar and cotton was used as a support material (Khan *et al*., 2001). The pH of the media was adjusted to 5.6-5.7 and then sterilized in an autoclave. The sterilized leaves were cut into discs of approximately equal sizes (~1 inch^2) and were placed on to the media in a way that abaxial surface of the leaf disc was down on the medium. Each media formulation was inoculated by 10 discs (2 discs per jar). All the jars were placed under illumination, provided by white fluorescent tube lights (~2000 Lux), with a photoperiod of 16 hours. Callus induction rate, on each media formulation, was calculated. Data were recorded after every week, for four weeks and the values presented as means ± standard deviation.

Shoot induction and multiplication: Shoot induction and multiplication from calli were evaluated by using different combinations of NAA and BAP with MS media (Table 2). All the media formulations had 2.5% of sugar and 2.5 gm/L of phytagel as a solidifying agent. The media were sterilized in an autoclave with a pH value of 5.6-5.7 before autoclaving. Fifteen jars, of each media formulation, were inoculated by the induced callus. Data were recorded weekly on Shoot Regeneration, No. of Shoots and Shoot Length for six weeks and values are the means ± standard deviation.

Root induction: The *In vitro* rooting in African violet was studied using MS media with different types of auxins like NAA, IAA, IBA and 2,4-D. Each auxin was used in three different concentrations (1.0, 2.0 and 3.0 mg/L). A simple MS medium, without any PGR, was used as a control. All the media formulations for root induction had 2.5% of sugar and 2.5 gm/L of phytagel as a solidifying agent. The media were sterilized in an autoclave, with a pH value of 5.6-5.7 before autoclaving. Fifteen explants (obtained from the shoot induction and multiplication experiment) were inoculated to each formulation. Data were recorded weekly for six weeks and values presented means ± standard deviation.

Acclimatization: Four different potting mixes were used to find out suitable potting mix for the *ex Vitro* growth of plantlets (Table 4). An equal number of plantlets was transferred to each potting mix and their survival rate was calculated after collecting data for three months (Table 4).

Table 1. Effect of auxins and their concentrations on the callus induction in African violet.

S. No.	Plant growth regulators	Media codes	Concentration (mg/L)	Callus induction (%)
1.	NAA	N1	1.00	95.00
		N2	2.00	94.00
		N3	3.00	95.00
2.	IBA	B1	1.00	65.00
		B2	2.00	68.00
		B3	3.00	70.00
3.	IAA	A1	1.00	65.00
		A2	2.00	72.00
		A3	3.00	75.00
4.	Control	C	XX	22.00

Table 2. *In vitro* shoot regeneration and multiplication in African violet at different media formulations.

S. No.	NAA (mg/L)	BAP (mg/L)	Media codes	Shoot regeneration (%)	Mean no. of shoots	Mean shoot length (cm)
1.	1	1	NB1	90.00	16.80 ± 0.32	2.2 ± 0.20
		2	NB2	90.00	18.40 ± 0.21	2.8 ± 0.22
		3	NB3	100.00	20.40 ± 0.22	5.0 ± 0.18
		4	NB4	100.00	19.80 ± 0.12	5.1 ± 0.21
		5	NB5	100.00	20.00 ± 0.14	5.1 ± 0.34
2.	2	1	NB6	85.00	17.10 ± 0.31	4.0 ± 0.22
		2	NB7	90.00	18.50 ± 0.21	3.8 ± 0.21
		3	NB8	100.00	20.20 ± 0.33	5.2 ± 0.10
		4	NB9	100.00	20.20 ± 0.14	5.1 ± 0.15
		5	NB10	100.00	20.30 ± 0.25	5.1 ± 0.19
3.	3	1	NB11	90.00	17.40 ± 0.24	4.2 ± 0.16
		2	NB12	100.00	19.10 ± 0.36	4.8 ± 0.07
		3	NB13	100.00	20.40 ± 0.22	5.1 ± 0.19
		4	NB14	100.00	20.30 ± 0.34	5.4 ± 0.12
		5	NB15	100.00	21.00 ± 0.32	5.5 ± 0.15

*Values are Means ± Standard Deviation (SD).

Results and Discussion

A highly efficient protocol for the callus induction, plantlet regeneration and acclimatization of African violet (*Saintpaulia ionantha*) was established. The protocol is cost effective in terms of chemicals as two of the high value chemicals, sucrose and agar, were replaced by low value sugar and cotton, respectively.

Callus induction: Three different concentrations of auxins, like NAA, IBA and IAA, were used to study their effects on the callus induction. In the light of the results obtained, NAA @ 1, 2 and 3 mg/L proved most efficient for the induction of callus (Table 1). Since the callus induction rate, in all the three concentrations of NAA, was not significantly different therefore it is suggested to use the lowest concentration of NAA in order to avoid any chance of somaclonal variation at the time of regeneration. The other two hormones i.e., IAA and IBA were not as much efficient as NAA although they were capable to produce calli with a respectable rate. The control treatment i.e., MS medium without any hormone, was capable to induce callus only in a trace amount. In this study, leaf was found effective as an explant source. Previous reports (Sunpui & Kanchanapoom, 2002) however recommended to use petiole as explant source.

Table 4. Effect of different potting mixes on the acclimatization of *In vitro* grown plantlets of African violet.

S. No.	Potting mix (v/v)	Code	Survival rate (%)
1.	100 % Sand	A1	95%
2.	50% Sand + 50% Grinded Charcoal	A2	65%
3.	50% Sand + 50% Coconut Husk	A3	70%
4.	50% Sand + 50% Farmyard Manure	A4	80%

Fig. 1. Different stages of micropropagation of African violet (*Saintpaulia ionantha*). (A) Shoot multiplication. (B) Root induction. (C) Acclimatization.

Shoot induction and multiplication: Different combinations of an auxin (NAA) and a cytokinin (BAP) were used for the regeneration experiment. Maximum regeneration (100%) was observed when 1.0 mg/L of NAA was used in combination with 3.0 mg/L of BAP (NB3). The same formulation also proved suitable for the shoot multiplication as maximum number and length of shoots were observed on the same formulation (Table 2). Other combinations of NAA and BAP were capable of producing more or less the same results (Table 1), but the lowest concentrations of both hormones are recommended to reduce the overall cost of the protocol. Mithila *et al*., (2003) reported the use of Thidiazuron (TDZ) for the induction of shoots in African violet however sufficient number of shoots was regenerated without the use of TDZ in this study.

Root induction: *In vitro* induction of roots in *Saintpaulia ionantha* was studied by using various concentrations of four different types of auxins viz., NAA, IBA, IAA and 2,4-D with a control (simple MS medium). All three concentrations of the four hormones were capable to induce roots in the regenerated plantlets alongwith the induction of some callus. Surprisingly, the control medium (without hormone), was found sufficient for the induction of roots as the number of roots induced by the control was much higher than the media which didn't induce calli (Table 3). Similar response was observed for *Kalanchoe tomentosa In vitro* root induction (Khan *et al*., 2006). These results might be due to the fact that the *in vitro* grown plantlets of *Saintpaulia ionantha* produced sufficient amount of auxins endogenously and no exogenous auxins was required for the induction of roots.

Acclimatization: The sufficiently rooted plantlets were then transferred to the green house for acclimatization. In this experiment four different types of potting mixes were used (Table 4). The health of the plantlets was observed weekly and the survival rates of the *In vitro* grown plantlets were calculated after three months. A very high survival rate (95%) was observed when 100 % garden sand was used as a potting mix (Table 4). A relatively high survival rate was also observed in the case of 50% sand with 50% farmyard manure. The respective survival rates of the plantlets in different potting mixes are presented in (Table 4).

It was observed that although the plantlets were sufficiently rooted, it is nearly mandatory for the plantlets of all the potting mixes that they should be kept in the tunnel covered with the plastic sheet, prior to the direct exposure of the plantlets under green house conditions. The time span required by the plantlets in the tunnel varied with the season. In summer (from March to August), the plantlets should be kept in the tunnel for at least 2 months whereas in winter (from September to February), that time period becomes shorter i.e. approximately 3 weeks. In the light of these observations, it can be safely said that the best time for the transfer of plantlets for acclimatization is winter season.

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