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Plant Regeneration and Floral Bud Formation from Intact Floral Parts of African Violet (*Saintpaulia ionantha* H. Wendl.) Cultured *in vitro*

N. Daud and R.M. Taha
Institute of Biological Sciences, Faculty of Science, University of Malaya,
50603 Kuala Lumpur, Malaysia

Abstract: Intact immature flower buds of African violet (*Saintpaulia ionantha* H. Wendl.) were used as explant sources for *in vitro* studies. The effect of exogenous hormones, NAA and BAP on the indirect organogenesis of this species was observed. Callus was formed on the cut end (base) of pedicels of floral buds where they were in contact with the medium. When maintained on the same medium, callus was differentiated into adventitious shoots after 10 weeks in culture. MS media supplemented with 2.0 mg L^{-1} NAA and 1.0 mg L^{-1} BAP gave the highest number of sterile or vegetative floral buds from the surface of callus of the explants, but these buds failed to develop further. The floral buds were expanded as abnormal flowers. The floral structures were smaller in size compared to intact flowers. Petals (corolla) were white to purple in colour but did not form any reproductive organs i.e., stamens or pistils. All sterile or vegetative floral buds and abnormal flowers survived for 3 months in culture but failed to reach anthesis.

Key words: *Saintpaulia ionantha*, *in vitro*, organogenesis, adventitious shoots, vegetative floral buds

INTRODUCTION

Saintpaulia ionantha or known worldwide as African violet (Moore, 1957) belongs to the family Gesneriaceae. This plant was described as a new species by Wendland in 1892 and was discovered in Tanga, East Africa by Paul (Kimmins, 1980; Heywood, 1996). African violet is considered to be one of the most popular ornamental indoor plants. They offered a health of beautiful flowers. There are many varieties of cultivated African violet and new hybrids with different flower colours are being developed.

Many previous reports stated that African violet has high capacity for *in vitro* regeneration and consequently, great potential for mass propagation. This species can regenerate via organogenesis or somatic embryogenesis. Organogenesis *in vitro* involves the formation of adventitious organs from areas of active cell division. Adventitious organs arise either directly from the original tissue (direct organogenesis) or indirectly through a callus phase (indirect organogenesis) (George and Sherrington, 1984).

A lot of reports have been published on regeneration of African violet in tissue culture such as from vegetative parts, i.e., leaf segments (Redway, 1990; Lo, 1997; Cassells and Plunkett, 1984; Cassells *et al*., 1986; Start and Cumming, 1976; Jain, 1993) petioles (Bilkey *et al*., 1978; Harney and Knop, 1979) and from floral parts (Molgaard *et al*., 1991; Vasquez and Short, 1978; Weatherhead *et al*., 1982). Adventitious shoots can be obtained from floral parts such as ovary, sepal and petal tissues (Molgaard *et al*., 1991). Bigot (1974) reported that most of organogenesis *in vitro* has been initiated from floral parts for many varieties of plants species. Adventitious shoot buds have been produced directly from the surface of cultured peduncles, for instance in *Gloxinia hybrida*.

The present study reports complete plant regeneration and formation of floral buds in African violet, from immature intact flower buds cultured on MS medium supplemented with various concentrations of NAA and BAP. However, the regeneration was achieved indirectly from callus phase which later differentiated to give rise to adventitious shoots and ultimately developed into complete plants.

MATERIALS AND METHODS

Immature flower buds of African violet (*Saintpaulia ionantha* H. Wendl.) were excised from 2-month-old intact plants which were grown in greenhouse. The flower buds (3-5 mm) were used as explants for *in vitro* studies. They were surfaced sterilized by immersing in 5% (w/v) Teepol solution for 1 min

Corresponding Author: Prof. Dr. Rosna Mat Taha, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia Tel: 603-79674372 Fax: 603-79674173

followed by 70% (v/v) ethanol for 30 sec and then in a 7% (v/v) chlorox for 5 min. They were then rinsed three times in sterile distilled water. After the last wash, the explants were ready to be cultured on MS medium (Murashige and Skoog, 1962) with 0.8% (w/v) agar (Sigma) and 3% (w/v) sucrose. The medium was supplemented with different concentrations of NAA, BAP and combinations of NAA and BAP (0 to 2.0 mg L^{-1}). Flower buds were cut including some parts of pedicel. The basal end of the flower buds were placed with 2-3 mm its length embedded in the medium. The medium was autoclaved for 21 min at 121°C after adjusted the pH to 5.8. Cultures were maintained at culture room condition of light intensity 1000 lux, temperature of 25±2°C and 70-80% relative humidity with a 16 h light and 8 h dark photoperiod. Twenty five explants were cultured. The formation of flower buds was examined and scored.

RESULTS AND DISCUSSION

The immature floral buds of African violet cultured on MS medium supplemented with hormones NAA and BAP showed indirect organogenesis and managed to regenerate adventitious shoots or vegetative floral buds. In this experiment, the floral buds were formed *in vitro* with sepal (calyx) and petal (corolla) but did not show any formation of reproductive organs (stamens or pistils). The expanded flower has white to purple coloured corolla.

Initially, the wounded end (base) of pedicels of floral buds became swollen and became dark purple after three weeks in culture. Callus was also induced at the base of the pedicel when cultured on MS medium supplemented with 0.5-1.0 mg L^{-1} NAA and 0.5-2.0 mg L^{-1} BAP. The compact callus formed was creamy green in color. The amount of callus increased over a 5-6 weeks growth period and eventually most of pedicels turned into callus (Fig. 1). Upon subculture onto the same media, this callus differentiated into adventitious shoots. The shoots became elongated after transferred to MS basal medium for 4 months, but the floral buds failed to open. These results are contrast with African violet of other cultivars, which was found possible to develop adventitious shoots in floral parts. For instance, when the whole inflorescence of African violet cultivar Valencia was placed in culture medium, shoots regenerated directly from the bract axils after 5 weeks in culture (Lineberger and Drunckenbrod, 1985).

In this study, vegetative floral buds appeared either directly from pedicel segment or from callus when the explants were cultured on MS medium containing various concentrations of NAA and BAP (1.5-2.0 mg L^{-1} NAA and 0.5-2.0 mg L^{-1} BAP) within 2 months in culture (Fig. 2). Some callus was formed at the base of pedicel. However, these floral buds could not develop further *in vitro*. The numbers of floral buds that were formed depended on the type and concentrations of hormones. In the present experiment, all floral buds when subcultured onto the same medium, survived for 3 months in culture. The floral bud structures only expanded when cultured on MS medium containing 2.0 mg L^{-1} NAA and 1.0 mg L^{-1} BAP. However, the flower was smaller than the intact flower and the petals (corolla) were white to purple in colour. There were no reproductive organs such as stamens and pistils that were formed in culture (Fig. 3). According to Nitsch and Nitsch (1967), the buds with bract but devoid of flowers have been termed as vegetative inflorescence.

The present results may be due to the auxin and cytokinin effect which is actually indirect. The exogenous

Fig. 1: The callus was creamy green in colour and compact at the base of floral bud

Fig. 2: Vegetative floral buds developed at the base of pedicel within 2 months in culture. Some callus was also formed at the base of pedicel

Table 1: The effect of BAP alone and in combinations of NAA and BAP on the formation of adventitious shoots and floral buds of African violet explants after three months in culture

Hormone concentration (mg L^{-1})		Explants with adventitious shoots (%)	No. of adventitious shoots per explant	Explants with vegetative floral buds (%)	No. of vegetative floral buds per explant
NAA	BAP				
0.5	0.5	40.0±0.5	1.7±2.2	0	0
0.5	1.0	32.0±0.5	1.5±2.1	0	0
0.5	1.5	40.0±0.5	1.6±2.1	0	0
0.5	2.0	52.0±0.5	2.2±2.2	0	0
1.0	0.5	100.0	4.1±1.0	0	0
1.0	1.0	100.0	5.4±2.2	0	0
1.0	1.5	60.0±0.5	3.2±4.5	0	0
1.0	2.0	60.0±0.5	2.8±3.9	0	0
1.5	0.5	40.0±0.5	1.8±4.0	0	0
1.5	1.0	0	0	60.0±1.2	1.2±1.2
1.5	1.5	0	0	40.0±0.5	1.0±1.3
1.5	2.0	0	0	20.0±0.4	0.6±1.2
2.0	0.5	0	0	40.0±0.5	0.9±1.2
2.0	1.0	0	0	76.0±0.4	2.2±1.6
2.0	1.5	0	0	56.0±0.5	1.4±1.5
2.0	2.0	0	0	20.0±0.4	0.4±0.9

Twenty-five replicates were used for each treatment. Mean Standard error (Mean±SE) is indicated

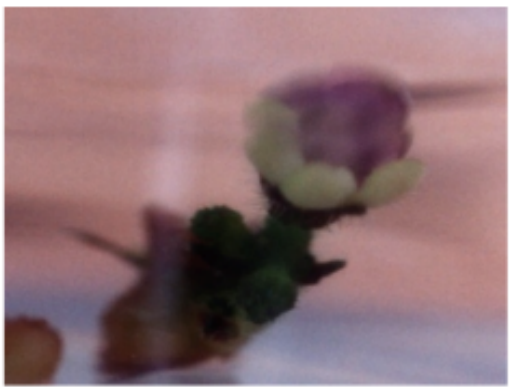

Fig. 3: The floral structure of African violet is characterized by corolla with white to purple color. No reproductive organs i.e., stamens or pistils were formed in culture

hormones, both NAA and BAP with different concentrations were required for callus to differentiate and subsequently to develop adventitious shoots or adventitious floral bud structures (Table 1). All floral structures were originated from somatic cells. Both auxin and cytokinin probably have roles to play in the floral development processes. However, auxin did not stimulate floral bud formation from leaf explants of *Begonia* (Ringe and Nitsch, 1968). In the case of *Plumbago indica* (Nitsch and Nitsch, 1967) the medium supplemented with auxin and cytokinin further increased reproductive bud formation. According to Nitsch and Nitsch (1967), optimal reproductive buds formation seems to occur when the NN basal medium (Nitsch and Nitsch medium) were supplemented with auxin, cytokinin and adenine.

According to Scorza (1982), the production of adventitious floral structures has been reported from various explants of several species. These structures are formed either from direct or indirect organogenesis. Peeters *et al*. (1991) reported that cytokinin was important for initiation of floral buds for *Rhododendron* sp. whereas, auxin induced floral bud differentiation from thin layer of pedicel explants of *Nicotiana* sp. In contrast, the previous reports on African violet with vegetative parts such as leaf and petiole segments (Cassells and Plunkett, 1984; Start and Cumming, 1976; Harney and Knap, 1979) did not show any similar adventitious floral bud formation. It was suggested that specific gene for flowering are activated during adventitious bud organogenesis. In this case, the induction of floral buds occurred in culture, when the explants were taken from plants which had already been flowering. Thus, cells competent for floral buds structure formation were present on floral explants but not on leaf and petiole explants.

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