ORIGINAL ARTICLE

Plant regeneration from petiole and leaf of African violet (*Saintpaulia ionantha* Wendl.) cultured *in vitro*

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Abstract

Sunpui, W. and Kanchanapoom, K.
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Songklanakarin J. Sci. Technol., 2002, 24(3) : 357-364

A regeneration procedure was developed for *in vitro* grown African violet (*Saintpaulia ionantha* Wendl.). Adventitious shoot regeneration was compared among petioles, leaves and stems cultured on MS basal medium. High frequency regeneration was obtained from leaves and petioles supplemented with plant growth regulators (BA, KN, 2,4-D, and NAA). Regeneration media that induced the highest number of shoots in petiole explants was either MS medium supplemented with 3 mg/l BA alone or 1 mg/l NAA plus 3 mg/l BA. Adventitious shoots were also regenerated from callus that was derived from leaf explants cultured on MS medium containing several concentrations of TDZ. Callus induced on medium with 0.5 mg/l TDZ showed the best shoot induction. Regenerated shoots could be rooted on $^{1}/_{2}$MS medium or MS medium supplemented with 3 mg/l IBA. Complete plantlets were acclimatized and successfully transplanted to glasshouse conditions.

Key words : adventitious shoot, cytokinins, organogenesis, regeneration, *Saintpaulia ionantha*

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Received, 3 April 2002 Accepted, 7 June 2002

บทคัดย่อ

วิชาดา สุ่นปุย และ คำนูณ กาญจนภูมิ
การเกิดพืชจากการเพาะเลี้ยงก้านใบและใบของต้นแอฟริกันไวโอเล็ต (*Saintpaulia ionantha* Wendl.) ในหลอดทดลอง
ว. สงขลานครินทร์ วทท. 2545 24(3) : 357-364

ได้พัฒนาการเพาะเลี้ยงเนื้อเยื่อของต้นแอฟริกันไวโอเล็ต (*Saintpaulia ionantha* Wendl.) ในหลอดทดลอง โดยการคัดเลือกชิ้นส่วนใบ ก้านใบ และลำต้นของต้นแอฟริกันไวโอเล็ตในอาหารสังเคราะห์สูตรพื้นฐาน MS ปรากฏว่า ชิ้นส่วนใบและก้านใบให้ความถี่ในการเกิดยอดสูง โดยเกิดยอดรวมได้ในอาหารสูตร MS ที่เติมสารควบคุมการเจริญเติบโตของพืชหลายชนิดได้แก่ BA, KN, 2,4-D และ NAA สูตรอาหารที่สามารถชักนำให้เกิดยอดรวมจากชิ้นส่วนก้านใบสูงสุดคืออาหารสูตร MS เติม BA 3 มก/ล เพียงอย่างเดียว หรือ NAA 1 มก/ล ร่วมกับ BA 3 มก/ล เมื่อนำชิ้นส่วนใบมาวางเลี้ยงบนอาหารสูตร MS ที่มี TDZ ความเข้มข้นต่าง ๆ สามารถชักนำให้เกิดแคลลัสและเจริญพัฒนากลายเป็นยอดรวมได้ โดยที่ความเข้มข้น TDZ 0.5 มก/ล เกิดยอดรวมมากที่สุด นำยอดที่ได้มาชักนำการสร้างรากบนอาหารสูตร $^1/_2$ MS หรือบนอาหารสูตร MS ที่มี IBA 3 มก/ล สามารถชักนำให้เกิดรากได้ดี ต้นแอฟริกันไวโอเล็ตที่สมบูรณ์สามารถเจริญเติบโตได้ดีในเรือนกระจก

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Saintpaulia ionantha Wendl., commonly known as African violet or *Saintpaulia*, belongs to the family Gesneriaceae. This species contains many cultivars with varied color and shape and it is a popular commercial plant. 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 year round (Grout, 1990). To date, micropropagation techniques are employed to produce a large number of new and true-to-type plants in a relatively short period of time. *In vitro* culture of *Saintpaulia* has been successfully initiated from several sources of explants including leaf (Start and Cumming 1976; Cooke, 1977; Smith and Norris, 1983), flower bud (Molgaard *et al*., 1991), subepidermis (Bilkey and Cocking 1981), anther (Weatherhead *et al*., 1982), protoplast (Hoshino *et al*., 1995; Winkelmann and Grunewaldt, 1995). Recent development in somatic hybridization and direct gene transfer are techniques of interest to extend the genetic variability in *Saintpaulia*. To establish a gene transfer system, it is essential to increase the rate of regeneration beyond these values. In this paper, we describe efficient direct shoot regeneration from leaf and petiole explants suitable for genetic transformation.

Materials and Methods

Maintenance of shoot cultures

Experiments were performed using *in vitro* grown *Saintpaulia ionantha* Wendl. shoots. Plant cultures were maintained by subculturing shoots at 4-week intervals onto the basal medium of Murashige and Skoog (MS, 1962) supplemented with 3% sucrose and 1 mg/l BA, gelled with 0.15% Gelrite (Merck & Co., Kelco Division, NJ, USA). Cultures were incubated at 25-27°C under a16:8 h photoperiod. The light source was Gro-Lux light providing 20 $\mu molm^{-2}s^{-1}$ photosynthetic photon flux density at the culture level.

Regeneration experiments

Regeneration experiments were conducted in 115-ml glass jars capped with a polystyrene screw cap containing a volume of 20 ml of nutrient medium. The medium was sterilized by autoclaving at 1.05 kg/cm^2 at 121°C for 20 min.

The various plant growth regulators supplement included NAA, BA, IBA, KN, 2,4-D, and TDZ. One explant was planted per jar and all experiments were carried out with 20 cultures per treatment. All experiments were repeated on three different days. Cultures were observed and adventitious shoots counted for up to 60 days.

Petiole experiments

Petiole segments were cut 3-5 mm long and placed with the basal cut surface down on the medium. The responses of petiole to one auxin (NAA) and two cytokinins (BA and KN) at concentrations 1, 2 and 3 mg/l were examined. In more comprehensive experiments, the interaction of 1, 2 and 3 mg/l NAA in combination with 1, 2 and 3 mg/l BA was investigated.

Leaf experiments

Leaf blades of length 0.5-1 cm were placed with the abaxial surface down on the medium. Two different auxin types, namely NAA at concentrations 1,2 and 3 mg/l and 2,4-D at concentrations 0.1, 0.3, 0.5, 0.7, 1, 2, and 3 mg/l and one cytokinin (TDZ) at concentrations 0.1, 0.5 and 1 mg/l were employed individually.

All regenerated shoots were transferred to hormone-free MS basal medium, half strength MS basal medium ($^1/_2$MS) and MS medium supplemented with 1-3 mg/l IBA for root induction.

Results

Plant regeneration from leaves and petioles

Various explants for their capacity to regenerate shoots were initially screened. Leaf, petiole and stem explants were cultured on MS basal medium devoid of growth regulators. There were differences in average number of shoots among the sources of explants (Table 1). The leaf explants regenerated shoots more effectively than petiole explants. Both explants showed green shoot primordia after 2-3 weeks of culture. These primordia developed into adventitious shoots after 4-5 weeks. However, most leaf explants became pale and necrotic over a 6-8 week period. In contrast to leaf and petiole explants, stem had very poor regeneration abilities (Table 1). The petiole explants appeared to be superior to the other two explants and thus used in the subsequent experiments.

Petiole experiments

With BA and KN incorporated in the media, adventitious shoots were formed after 8 weeks. Comparison of shoot regeneration from petiole explants on BA- and KN- containing media showed that the number of shoots produced per explant was dependent on growth regulators and BA was more effective than KN (Table 2). Increase of BA and KN enhanced the number of shoot regeneration. The highest number of shoot produced was

Table 1. Comparison of shoot regeneration efficiency from various explants. Regenerated shoots were scored after 4 weeks of culture on MS basal medium.

Explants	Shoot regeneration (%)	Number of shoots per explant* ($\bar{X} \pm$ SD)
Stem	90	$6.60^c \pm 1.23$
Petiole	100	$31.25^b \pm 2.21$
Leaf	100	$39.20^a \pm 1.79$

*** Each value represents the mean number (± standard deviation) of three independent experiments. Means followed by different letters are significantly different at the 95% confidence level.**

Table 2. Effect of BA, KN and NAA at different concentrations on number of adventitious shoots regenerated on petiole explant of *Saintpaulia*.

Concentration of growth regulator (mg/l)	Mean number of adventitious shoots for* BA	KN	NAA
0	$33.45^{d} \pm 1.67$	$33.45^{d} \pm 1.67$	$33.45^{d} \pm 1.67$
1	$46.45^{c} \pm 3.86$	$42.00^{c} \pm 2.20$	C
2	$61.05^{b} \pm 1.70$	$45.90^{b} \pm 1.37$	C
3	$83.85^{a} \pm 1.81$	$70.55^{a} \pm 1.64$	C

The data were recorded after 8 weeks of culture and represent the mean number of shoots per explant ± standard deviation.

*** Values represent the mean number of three independent experiments. Means followed by different letters are significantly different at the 95% confidence level.**

C = callus formation

Table 3. Effect of BA and NAA at different combinations on number on adventi tious shoot regenerated on petiole explant of *Saintpaulia*. Regenerated shoots were scored after 8 weeks of culture.

Concentrations (mg/l) NAA	BA	Shoot regeneration (%)	Number of shoots per explant* $\overline{X} \pm SD$
1	1	100	$116.50^{b} \pm 1.82$
1	2	100	$123.05^{b} \pm 1.47$
1	3	100	$143.75^{a} \pm 1.41$
2	1	100	$79.10^{d} \pm 18.69$
2	2	100	$96.45^{c} \pm 1.88$
2	3	100	$107.75^{b} \pm 1.86$
3	1	85	$46.30^{e} \pm 20.00$
3	2	85	$34.25^{f} \pm 14.81$
3	3	75	$24.65^{f} \pm 14.66$

*** Each value represents the mean number (± standard deviation) of three independent experiments. Means followed by different letters are significantly different at the 95% confidence level.**

found in the media supplemented with 3 mg/l BA. A similar number of shoots were regenerated on media with KN. No callus was formed by the explants on the basal media with either BA or KN. On NAA-containing media (1-3 mg/l) encouraged callus formation with poor shoot proliferation.

Subsequent experiment was initiated to assess the effectiveness of petiole segments over a series of NAA-BA ratios in achieving regeneration. The data are presented in Table 3. With NAA and BA at all concentrations incorporated in the media, yellow green and friable callus was formed over the surface of the petioles. Small green nodules began to form on this callus within 4 weeks of culture. These nodules developed into buds and subsequent adventitious shoots were formed after a culture period 8 weeks (Figure 1). Multiple shoots obtained in all concentrations

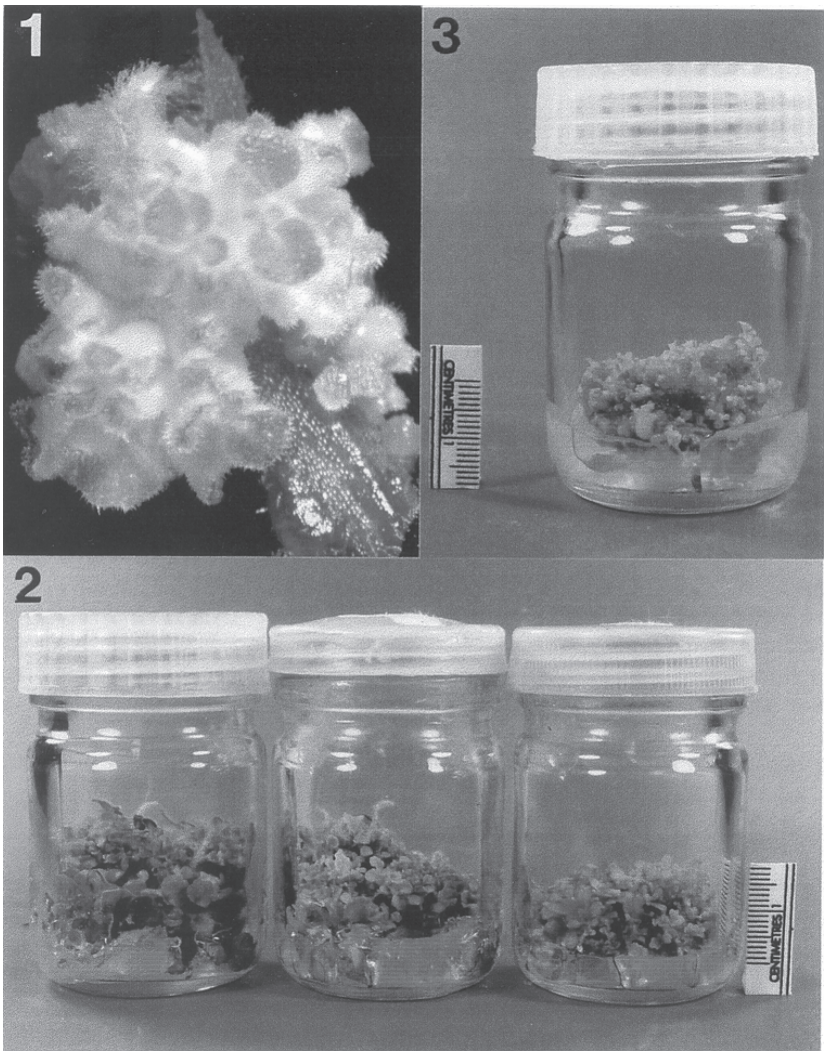

Figure 1-2. Adventitious shoot regeneration from a petiole-derived callus on MS medium containing 1 mg/l NAA and 3 mg/l BA (1) and 1 mg/l NAA in combination with 1, 2, and 3 mg/l BA (2, from left to right) 8 weeks after the initial culture.

Figure 3. Adventitious shoot regeneration from a leaf-derived callus on MS medium containing 0.5 mg/l TDZ after 3-4 weeks of culture.

showed more or less similar results with regard to the number of shoots produced per explant. Increasing NAA concentrations reduced shoot production as well as increased callus formation. No root formation was evident on these media. The highest number of shoot produced was found in the media supplemented with 1 mg/l NAA and 3 mg/l BA therefore this medium was selected for shoot multiplication (Figure 2). Shoots continued to grow rapidly while still attached to the original explant. The leaves of the adventitious shoots were healthy with green color and did not show any sign of vitrification.

Regenerated shoots could be excised into single shoots over several subcultures and transferred to root induction medium. Shoots obtained

from the shoot induction media did not produce roots hence single shoots of 1.5-2 cm in length were used for *in vitro* rooting. It appeared from our experiments that hormone-free MS basal medium, 1/2 MS and MS medium supplemented with1-3 mg/l IBA were effective for root induction (Table 4). More roots developed from the excised shoots on MS medium containing 3 mg/l IBA; however, roots that developed from 1/2 MS medium were healthy, thick, long and fibrous.

Leaf experiments

The earlier experiment using leaf cultured on hormone-free MS basal medium revealed that most leaf explants regenerated shoots effectively but became pale and necrotic over a 6-8 week period. Consequently, the effect of NAA, 2,4-D and TDZ were tested on leaf explants. On these tested media, shoot regeneration was not observed and callus formation was evident. After 1-2 weeks in the culture media (1, 2, and 3 mg/l NAA and 0.1, 0.3, 0.5, 0.7, 1, 2, and 3 mg/l 2,4-D) the leaf explants remained green and watery. At the fifth week of culture, the explants turned brown and small granulated callus formed at the margin of the leaves. Only a small percentage of calli were able to form on NAA- and 2,4-D-containing media. Most leaves were induced to form good callus on the MS media enriched with 0.1, 0.5, and 1 mg/l TDZ. In the beginning, callus growth was rather slow and after a 4-week culture period the speed of callus formation was fast and small green nodules could be seen. Eight weeks after initial cultures numerous adventitious shoot formation took place readily from these nodules. The number of adventitious shoots varied depending on the concentrations of TDZ (Table 5 and Figure 3).

Transfer to soil

Gelrite was carefully washed from all regenerated plants, which were then planted in sterile vermiculite until root systems were well established. They were then transferred to potting soil and normal looking *Saintpaulia* plants were obtained. The total duration of the cycle from either petiole or leaf explants through complete plantlets was 14 weeks; 8 weeks for shoot initiation and multiplication, 4 weeks for root induction and development and 2 weeks for hardening.

Discussion

BA strongly enhanced regeneration of adventitious shoots in petiole explants of *Saint-*

Table 4. Rooting response of *in vitro* shoots of *Sainpaulia* as affected by medium types after 6 weeks of culture.

Medium type (mg/l)	Rooting (%)	Number of roots per shoot* ($\bar{X}$ ± SD.)
MS	100	29.55^{c} ± 1.54
$^{1}/_{2}$MS	100	40.25^{b} ± 1.55
MS + IBA 1	100	19.40^{e} ± 1.35
MS + IBA 2	100	25.30^{d} ± 1.81
MS + IBA 3	100	47.45^{a} ± 1.47

*** Each value represents the mean number (± standard deviation) of three independent experiments. Means followed different letters are significantly different at the 95% confidence level.**

Table 5. Effect of different concentrations of thidiazuron (TDZ) on adventitious shoot regeneration from leaf -derived callus cultured on MS medium for 8 weeks. The data represent the mean number of shoots per explant ± SD.

TDZ (mg/l)	Callusing (%)	Number of shoots* ($\bar{X}$ ± SD)
0.1	100	92.95^{b} ± 2.04
0.5	100	116.75^{a} ± 1.83
1.0	100	84.05^{c} ± 1.79

*** Each value represents the mean number (± standard deviation) of three independent experiments. Means followed by different letters are significantly different at the 95% confidence level.**

paulia, as it does in other species, such as carnation (Messeguer *et al*., 1993), gerbera (Nongmanee and Kanchanapoom, 1995), and gloxinia (Wuttisit and Kanchanapoom, 1995). The auxin/cytokinin balance is one of the factors with a determining effect on pattern of morphogenesis from petiole explants in order to increase the number of vegetative shoots. These combinations caused interactions that resulted in altered morphogenetic responses. Hence when NAA was used with BA, shoot bud regeneration increased markedly. There are many reports stated that TDZ was more effective in shoot regeneration than BA (Nugent *et al*., 1991; Nakano *et al*., 1994; Winkelman and Grunewaldt, 1995). *Saintpaulia* leaf explants seemed to have hardly any auxin requirement for callus growth. It is possible that endogenous growth substance content, presumably auxins, are synthesized in the shoots and translocate to the other parts such as leaves. This supply of auxin could be substantial thus with augmented supply of NAA and 2,4-D from the medium could be too high to maintain growth. The reproduction of *Saintpaulia* through callus is characterized by the presence of TDZ. TDZ has been used to induce shoot organogenesis in callus culture of *Phaseolus lunatus* (Mok and Mok 1985) and several woody species (Huetteman and Preece, 1993). High activities of TDZ in plant regeneration were also reported in other ornamentals such as carnation (Nakano *et al*. 1994), orchid (Chen *et al*. 1999) and roses (Hsia and Korban, 1996). We believe this is the first report on the use of TDZ for induction of shoot organogenesis in *Saintpaulia*. In our experiments the possible role of TDZ in shoot induction was shown conclusively on callus growth. Therefore, an extensive study with TDZ to find media for optimum shoot regeneration, particularly on petiole explants, should be further undertaken.

In conclusion this research demonstrated that direct plant regeneration of *Saintpaulia* through culture of petiole and leaf could be obtained easily. The rapid clonal propagation described here will be very useful for the development of gene transfer system and can be efficiently adapted for other ornamentals.

Acknowledgment

The Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla provided partial support for this work.

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