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Studies on Plant Regeneration and Somaclonal Variation in *Saintpaulia ionantha* Wendl. (African Violet)

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Abstract: Efficient plant regeneration of *Saintpaulia ionantha* (African violet) has been obtained in the present study. MS medium supplemented with 1.0 mg L^{-1} IAA and 2.0 mg L^{-1} Zeatin resulted in 100% shoot regeneration and induced the highest number of shoots (average 15.0±0.8 shoots per explant) after being cultured for 8 weeks. The above hormone combination was optimum for shoot regeneration. Most of *Saintpaulia ionantha* plantlets derived from tissue culture system could be hardened and transferred to the greenhouse conditions with 84.0±1.6% success rate. However, regenerated plantlets of *Saintpaulia ionantha* (even after 12 months old) failed to flower. Morphological characters of regenerated plantlets of *Saintpaulia ionantha* were observed and compared with *in vivo* (intact) plants. Regenerated plantlets showed some differences in morphological characters, such as height and leaf size, texture and colour, but the plantlets showed no variation in leaf arrangement and leaf margin. However, the morphological characters of the regenerated plantlets were found to be unstable.

Key words: Morphogenesis, *Saintpaulia ionantha*, organogenesis, somaclonal variation, plantlets, vegetative propagation

INTRODUCTION

Saintpaulia ionantha Wendl. commonly known as African violet belongs to the family Gesneriaceae. It consists of many cultivars with varied flower colour as well as leaf colour and shape. About 20,000 varieties have been produced globally by conventional breeding techniques. Several hundred new cultivars are released annually (Ghisleni and Martinetti, 1995). African violet is an excellent model system for *in vitro* regeneration studies because of its notable regenerative ability and accessibility (Lo, 1997). The development of healthy mother plants via tissue culture and their axenic true-to-type preservation has been reported (Jungnickel and Zaid, 1992). Similar to other plant species, growth regulators in culture medium are the major factor affecting shoot regeneration of African violet *in vitro*. As stated in many previous reports, African violet with high capacity for *in vitro* regeneration has the great potential to be used in organogenesis, involves the formation of adventitious organs from areas of active cell division. Adventitious organs arise either from the original tissue (direct organogenesis) or through a callus phase (indirect organogenesis) (George and Sherrington, 1884). Various reports had been published on regeneration of African violet in tissue culture such as from vegetative part, i.e., leaf segments (Cassells *et al*., 1986; George and Sherrington, 1984), petioles (Bilkey *et al*., 1978) and from floral parts (Vasquez and Short, 1982).

The previous reports also showed that shoot regeneration from various explants of African violet occurred on MS medium supplemented with various combinations of NAA and BAP, IAA and BAP and also combinations of IAA and Kinetin. Regeneration of shoots on medium containing NAA and BAP has been reported for other plants species such as *Dianthus caryophylla* L. (Vasquez and Short, 1982), *Sinningia allogophylla* (Bilkey *et al*., 1978) and *Begonia* (Fonnesbech, 1974). To date, there has been no published work on the effects of combinations of other auxin and cytokinin on regeneration of African violet, such as IAA and 2iP, IAA and Zeatin, NAA and 2iP, NAA and Kinetin, IBA and BAP, IBA and 2iP, IBA and Kinetin, IBA and Zeatin.

In vitro method of vegetative propagation such as adventitious shoot formation, callus culture, cell suspension culture, protoplast culture has the possibility

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for genetic variation and mutations (Buiatti *et al*., 1986). Somaclonal variation in plants is attributed to gene mutation, aneuploidy, transposable elements, genotype, type of explants used, age of the donor plant and type of plants hormones in the culture medium (Jain and Newton, 1990a, b). Unlike epigenetic changes, somaclonal variation which results from altered gene expression is usually irreversible (Karp, 1991, 1995). In the literature, it has been reported that used of some growth regulators such as NAA, 2,4-D, kinetin etc. can lead to increase number of mutation. High concentrations of kinetin in the medium were found to induce somaclonal variation in carrot (Ibrahim, 1969). Some of the differentiated plantlets had abnormally dissected leaves, which had intense green, thick and short with broad petioles. Repeated subcultures *in vitro* also increase the occurrence of variation for axillary and adventitious shoot formation. Somaclonal variation can result in changes in characters such as flower colour, number of flower per plants, leaves characters, pigmentation, etc. Plant regeneration via organogenesis or protoplasts often leads to more somaclonal variations (Jain and Newton, 1989; Karp, 1989, 1995). The length of culture period also influences somaclonal variation (Nehra *et al*., 1992). The objective of this study was to investigate the effect of hormone combinations for efficient regeneration from petiole segments of *Saintpaulia ionantha*. In this study, we report efficient plant regeneration on MS medium supplemented with various combinations of auxins and cytokinins.

MATERIALS AND METHODS

Plant materials: All experiments were conducted at Laboratory B2.5, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia in year 2003. In these experiments, explants used were petioles of *Saintpaulia ionantha* excised from aseptic seedlings (3-4 months-old). Excised petioles were surface sterilized with 10% Chlorox (Sodium hypochlorite) plus 2 drops of Tween 20 for 15 min and subsequently rinsed three times in sterile distilled water. After the last washing, the petiole segments were further cut into 5-10 mm pieces and ready to be cultured.

Regeneration medium and culture condition: After sterilization, petiole segments were cultured on MS medium (Murashige and Skoog, 1962) with 8.0 g L^{-1} Oxoid agar (Sigma) and 30.0 g L^{-1} sucrose. The medium was also supplemented with combinations of auxin (2,4-D, IBA, IAA and NAA) and cytokinin (BAP, 2iP, Kinetin and Zeatin). The medium was autoclaved for 21 min at 121°C after the pH was adjusted to 5.8. Cultures were maintained under the culture room condition of light intensity (1000 μmol m^{-2} sec^{-1}), temperature of 25±1°C and 70-80% relative humidity with a 16/8 h light/dark photoperiod.

Acclimatisation: Regenerated plantlets of *Saintpaulia ionantha* (4 months-old) were transferred into flowerpots containing soil mixture. The plantlets were placed under the culture room conditions of light intensity (1000 μmol m^{-2} sec^{-1}), a temperature of 25±1°C and 70-80% relative humidity with a 16/8 h light/dark photoperiod. For plantlet establishment, a high relative humidity was maintained under transparent plastic for 1 month. The plantlets were watered twice a week. Plant morphology (plant height and leaf shape) was the main criteria for selecting plantlets for propagation. The height of plantlets was measured with a scale. One month later, plantlets were transferred to the greenhouse and were retained to study growth and flowering. The variation of morphological characters observed in plantlets was recorded to study the somaclonal variation that could occur in the plantlets.

RESULTS AND DISCUSSION

Previous observations from this experiment on *Saintpaulia ionantha* showed that the ability for regeneration was not lost in cultured tissues or organs such as petiole, leaf, peduncle and petal on the hormone-free medium. Various reports also showed that petiole, leaf and stem segments of *Saintpaulia ionantha* were competent for shoot regeneration (approximately 90-100%) on MS basal medium (Sunpui and Kanchanapoom, 2002). The addition of hormones on the MS medium gave the best response to regenerate multiple shoots of various explants. Many researchers have reported on shoot differentiation from various explants of *Saintpaulia ionantha* on MS medium containing NAA and BAP and these combinations could be used as a common medium for most of the cultivars used (Cassells *et al*., 1986; Sunpui and Kanchanapoom, 2002; Vasquez and Short, 1982).

In preliminary experiment, the different combinations of NAA and BAP concentration (0-2.0 mg L^{-1} of range) were used based on the previous reports on *Saintpaulia ionantha* to estimate the optimum concentrations for regeneration. A screening test has been carried out with 24 combinations of NAA and BAP concentrations. Based on the results obtained, optimum concentration was

Table 1: Effects of various hormones, auxin (2,4-D, IBA, IAA and NAA) and cytokinin (BAP, 2iP, Kinetin and Zeatin) on shoot regeneration and number of shoots per explant after 8 weeks in culture (mean±standard deviation). Twenty-five petiole segments were cultured on MS medium supplemented with 1.0 mg L^{-1} auxin and 2.0 mg L^{-1} cytokinin

MS medium			
Types of auxin	Types of cytokinin	Regeneration (%)	No. of shoot/explant
-	-	80.00±0.09	6.80±1.70
IAA	BAP	100.00±0.00	6.50±0.45
IAA	2iP	100.00±0.00	4.00±0.42
IAA	Kinetin	90.00±0.10	4.10±0.87
IAA	Zeatin	100.00±0.00	15.00±0.80
NAA	BAP	100.00±0.00	12.85±0.25
NAA	2iP	100.00±0.00	3.50±0.48
NAA	Kinetin	70.00±0.16	2.70±0.54
NAA	Zeatin	-	-
2,4-D	BAP	100.00±0.00	Callus formation
2,4-D	2iP	-	-
2,4-D	Kinetin	100.00±0.00	Callus formation
2,4-D	Zeatin	-	-
IBA	BAP	70.00±0.16	3.00±0.74
IBA	2iP	100.00±0.00	5.60±0.61
IBA	Kinetin	70.00±0.16	1.20±0.41
IBA	Zeatin	100.00±0.00	5.20±0.56

found to be at 1.0 mg L^{-1} NAA and 2.0 mg L^{-1} BAP with petiole segments gave the best response to regenerate shoots (100% regeneration with average 12.85 shoots per explant) and more effective compared to leaf, petal and peduncle segments. This is in contrast with previous reports working on the same species. Sunpui and Kanchanapoom (2002) observed that the petiole segments gave 100% regeneration and higher number of shoots produced (123.0 shoots per explant) in the MS medium supplemented with 1.0 mg L^{-1} NAA and 2.0 mg L^{-1} BAP. The number of shoots produced were different, indicating that petiole explants were more responsive in culture, probably due to different in cultivars and related explant factors, such as physiological and physical in relation to size of explants, age of plantlets used and different culture conditions. Thus, petiole explants and combinations with concentrations of 1.0 mg L^{-1} auxin and 2.0 mg L^{-1} cytokinin were used in the subsequent experiments, with expectation that combinations of both auxin (IAA, NAA, 2,4-D, IBA) and cytokinin (Kinetin, Zeatin, BAP, 2iP) would induce more shoot regeneration. Table 1 shows the response of petiole explants from *Saintpaulia ionantha* with various combinations of other auxin and cytokinin. Shoots were formed on the cut surface of petioles cultured on MS medium supplemented with combination of hormones tested (Fig. 1). Shoot regeneration in all reports occurred directly, without callus stage (Fig. 2), indicating that the requirement for competence acquisition is not limited to callus formation.

Observation of shoot regeneration from petiole explants on MS medium containing auxin and cytokinin

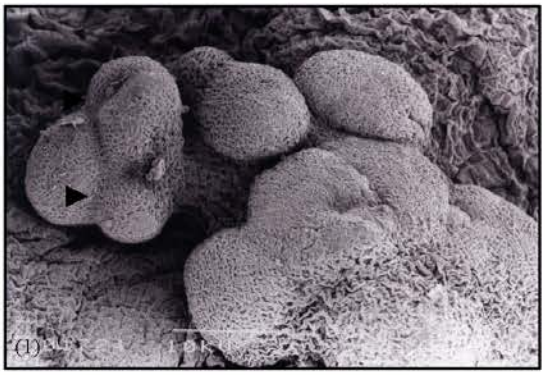

Fig. 1: Petiole explants of *S. ionantha* cultures on MS medium with 1.0 mg L^{-1} IAA and 2.0 mg L^{-1} Zeatin, showing the new meristem with leaf primordial

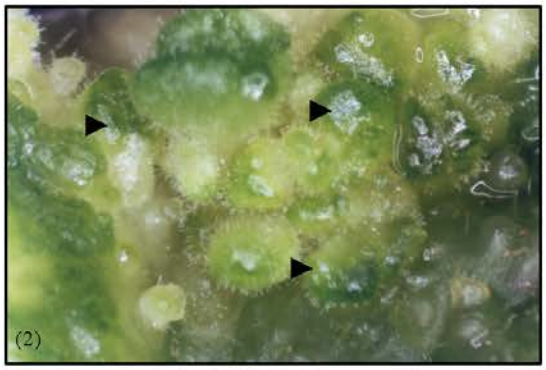

Fig. 2: Adventitious shoot buds were formed directly on the cut surface of petiole explants cultured for 6 weeks

(except of NAA and BAP) showed that the number of shoots produced per explant depended on hormones in the MS medium. MS medium supplemented with 1.0 mg L^{-1} IAA and 2.0 mg L^{-1} Zeatin gave 100% shoot regeneration and induced the highest number of shoots (average 15.0±0.8 of shoots for each explant) after 8 weeks in culture (Fig. 3). The number of shoots produced per explant of different combinations of hormones, i.e., 1.0 mg L^{-1} (IAA, NAA, IBA) and 2.0 mg L^{-1} (Kinetin, Zeatin, BAP, 2iP) were less compared to combinations of 1.0 mg L^{-1} IAA and 2.0 mg L^{-1} Zeatin. Combination of 1.0 mg L^{-1} IAA and 2.0 mg L^{-1} Zeatin was chosen as an optimum medium for further related experiments.

The different types of combination of auxins and cytokinins also was observed as one of the factors to determine the effects on morphogenesis pattern. In certain combinations tested, the response of morphogenesis

Fig. 3: Adventitious shoot regeneration from petiole segments on MS medium containing 1.0 mg L^{-1} IAA and 2.0 mg L^{-1} Zeatin after 8 weeks in culture

were callus formation and dwarf shoots. From our results, MS medium with combinations of 1.0 mg L^{-1} NAA and 2.0 mg L^{-1} Kinetin and also 1.0 mg L^{-1} IBA and 2.0 mg L^{-1} Kinetin produced dwarf shoots. Embryogenic callus was formed on the MS medium with combinations of 1.0 mg L^{-1} 2,4-D and 2.0 mg L^{-1} BAP and also 1.0 mg L^{-1} 2,4-D and 2.0 mg L^{-1} Kinetin. MS medium supplemented with combinations of NAA and Zeatin, 2,4-D and 2iP and 2,4-D and Zeatin failed to show any response. In this case, morphogenesis ability may be associated with endogenous hormones found in the explants. It is possible that endogenous auxin was synthesized in the shoots and translocated to other parts of plants such as petiole. This endogenous auxin could be substantial thus increased supply of 2,4-D or NAA from this medium.

Many reports stated that combinations of auxin and cytokinin were most effective on shoot regeneration of *Saintpaulia ionantha*, for instance combinations of IAA and BAP (Lo, 1997) and for other species such as 2,4-D and Kinetin on *Plumbago rosea* (Kumar and Bhavanandan, 1988) and also IBA and 2iP on *Rododendron* (Tomsone and Gerthnere, 2003). To our knowledge, this is the first report on the used of NAA-BAP combinations for induction of shoot regeneration in *Saintpaulia ionantha*.

Most of *Saintpaulia ionantha* plantlets derived from *in vitro* system can be hardened and transferred to the greenhouse conditions and would be well developed. However, from this study, regenerated plantlets of *Saintpaulia ionantha* failed to produce flowers. This probably was due to the type of explants used. In these experiment, plantlets derived from petiole explant cultures. Nitsch and Nitsch (1967) and De Klerk *et al*. (1990) stated that explants taken from plants that are already flowering have potential to produce flower buds in *in vitro* system.

Table 2: Macromorphology characters on *in vitro* plantlets of *Saintpaulia ionantha* (12 months old) in the greenhouse. Standard errors (Mean±SE) were indicated

Characteristics	*In vivo* plant (12 months old)	Regenerated plantlets (12 months old)
Height (mm)	18.0±0.4	17.8±0.2
Leaves		
Texture	Thick; a lots of hairy	Thick; a little hairy
Colour	Dark green; dull	Greenish; shiny
Shape	Elliptic to round	Elliptic
Margin	Undulate	Undulate
Arrangement	Rosette	Rosette
Size (3rd leaf from apex):		
(length; mm)	82.0±0.5	60.0±0.8
(broad; mm)	45.0±1.0	38.0±1.0

Fig. 4: Regenerated plantlet (6 months old) was grown in the greenhouse

Fig. 5: *In vivo* (intact) plant (12 months old) in the greenhouse

Morphological characters have been observed on regenerated plantlets (12 months old) and *in vivo* (intact) plants of *Saintpaulia ionantha* and summarized in Table 2. Regenerated plantlets (Fig. 4) compared with *in vivo* (intact) related plants (Fig. 5) showed some differences in morphological characteristics (phenotype) which were characterized by plant height, leaf characters

i.e., size, texture and colour. The leaf formed in regenerated plants was thick, little hairy, elliptic in shape, greenish shiny and small in size (Fig. 4). *In vivo* (intact) plants had showed that morphological (phenotype) characters such as thick leaf, lots of hairs, elliptic to round in shape, dark green colour, dull and medium in size (Fig. 5). However, the morphological characters demonstrated by regenerated plantlets were unstable. The leaf arrangements were rosette and leaf margin undulated, similar to *in vivo* (intact) plants. None of the plantlets demonstrated variation in leaf arrangement and margin, which seems to be quite stable in tissue culture condition

Somaclonal variations observed from regenerated plantlets were either due to hormones, culture medium or physical conditions. It is difficult to conclude that hormones or its interaction with other components in the culture medium causes the somaclonal variation. Somaclonal variation and other plant characteristics may be depending on the number of successive subcultures of *Saintpaulia ionantha* on the fresh medium before plant regeneration. De Klerk *et al.* (1990) suggested that the variation in micropropagated plants of *Begonia* x *hiemalis* of quantitative trait in leaf shape could be due to either somaclonal variation or epigenetic variation. The plant genotype can also cause somaclonal variation (Larkin and Scowcroft, 1981).

CONCLUSION

The high production of plantlets was observed on MS medium containing 1.0 mg L^{-1} IAA and 2.0 mg L^{-1} Zeatin which offers an alternative combination of hormones for micropropagation of this species. Furthermore, variations in some characters obtained could produce a new plant variety. Generally, based on our results, it is proven that tissue culture system of *Saintpaulia ionantha* would be useful for producing new variants of horticultural interest.

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REFERENCES

Bilkey, P.C., B.H. McCown and A.C. Hildebrandt, 1978. Micropropagation of African violet from petiole cross sections. Sci. Hortic., 13 (1): 37-38.

Buiatti, M., F. Gimelli, R. Venturo, P. Bogani and P. Picconi, 1986. Interclonal Variability Induced *in vitro* and *in vivo* Propagation in a Vegetatively Propagated Plant, the Carnation. In: Somaclonal Variation and Crop Improvement, Semal, S. (Ed.). Martinus Nijhoff Publishers Derdrcht (NL), pp: 251-256.

Cassells, A.C., A. Plunket and D. Kelleher, 1986. Screening of *Saintpaulia ionantha* Wendl. cultivars for caulogenetic potential based on the *in vitro* responses of young axenic leaves on auxin and cytokinin factorial media. Sci. Hortic., 30 (1): 151-157.

De Klerk, G.J., T.J. Brugge and H. Bouman, 1990. An assay to measure the extent of variation in micropropagated plants of *Begonia* x *hiemalis*. Acta Bot. Neerl., 39 (1): 144-151.

Fonnesbech, M., 1974. The influence of NAA, BA and temperature on shoot and root development from *Begonia* x *cheimantha* petiole segments grown *in vitro*. Physiol. Plant, 32 (1): 49-54.

George, E.F. and P.D. Sherrington, 1984. Plant propagation on Tissue Culture. Handbook and Directory of Commercial Laboratories, Exegetics Ltd., London, pp: 102-110.

Ghisleni, P.L. and L. Martinetti, 1995. Saintpaulia. In: Annali Accademia di Agricoltura di Torino, pp: 158-266.

Ibrahim, R.K., 1969. Normal and abnormal plants from carrot root tissue cultures. Can. J. Bot., 47 (5): 825-826.

Jain, S.M. and R.J. Newton, 1989. Proto-Variation in Protoplast Derived *Brassica napus* Plants. In: Progress in Plant Protoplast Research, Puite, K.J., J.J.M. Dons, H.J. Huizing, A.J. Kool, M. Koornneef and F.A. Krens (Eds.). Dordrecht: Kluwer Academic Publications, pp: 403-404.

Jain, S.M. and R.J. Newton, 1990a. Evaluation of protoclonal variation versus chemically induced mutagenesis in *Brassica napus* L. Curr. Sci., 58 (4): 176-180.

Jain, S.M. and R.J. Newton, 1990b. Prospects of biotechnology for tea improvement. Proc. Indian Natl. Sci. Acad, B 56 (15): 441-448.

Jungnickel, F. and S. Zaid, 1992. Micropropagation of African Violets (*Saintpaulia* spp. and cvs.). In: Biotechnology in Agriculture and Forestry, Bajaj, Y.P.S. (Ed.). Springer-Verlag, Berlin, 20: 357-395.

Karp, A., 1989. Can genetic instability be controlled in plant tissue cultures? IAPTC Newslett., 58: 2-11.

Karp, A., 1991. On the Current Understanding of Somaclonal Variation. In: Oxford Surveys of Plant Molecular and Cell Biology, Miflin, B.J. (Ed.). Oxford University Press, 7: 1-58.

Karp, A., 1995. Somaclonal variation as a tool for crop improvement. Euphytica, 85 (1): 295-302.

Kumar, K.S. and K.V. Bhavanandan, 1988. Micropropagation of *Plumbago rosea* Linn. Plant Cell Tissue Org. Cult., 15 (3): 275-278.

Larkin, P.J. and W.R. Scowcroft, 1981. Somaclonal variation-a novel source of variability from cell cultures for plant improvement. Theor. Applied Genet., 60 (4): 197-214.

Lo, K.H., 1997. Factors affecting shoot organogenesis leaf disc culture of African violet. Sci. Hortic., 72 (1): 49-57.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15 (3): 473-497.

Nehra, N.S., K.K. Kartha, C. Stusnoff and K.L. Giles, 1992. The influence of plant growth regulator concentrations and callus age on somaclonal variation in callus culture regenerants of strawberry. Plant Cell Tissue Org. Cult., 29 (3): 257-268.

Nitsch, C. and J.P. Nitsch, 1967. The induction of flowering *in vitro* in stem segments of *Plumbago indica* L. II. The production of reproductive buds. Planta, 72 (4): 371-384.

Sunpui, W. and K. Kanchanapoom, 2002. Plant regeneration from petiole and leaf of African violet (*Saintpaulia ionantha* Wendl.) cultured *in vitro*. Songklanakarin J. Sci. Technol., 24 (3): 357-364.

Tomsone, S. and D. Gerthnere, 2003. *In vitro* shoot regeneration from flower and leaf explants in *Rhododendron*. Biol. Plant., 46 (3): 463-465.

Vasquez, A.M. and K.C. Short, 1982. Morphogenesis in cultured floral parts of African violet. J. Exp. Bot., 29 (5): 1265-1271.