Variations in African Violet 'Crimson Frost' Micropropagated by Homogenized Leaf Tissue Culture

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SUMMARY. Adventitious buds regenerated from homogenized leaf tissue of *Saintpaulia ionantha* Wendl. 'Crimson Frost' were micropropagated to determine types and frequencies of the variants obtained. Plants grown for one year in a greenhouse showed 67% variation and 33% normality. A higher rate of variation was observed in leaf color rather than in leaf shape. The variations in leaf color and leaf shape were 67% and 19%, respectively. In regard to flower type, greater numbers of semidouble and double types were obtained as compared to single types. Both flower types showed a much higher rate of normal (mixed) color (81%) as compared to pink, red or white (19%). An sodium dodecylsulfate polyacrylamide gel electrophoresis profile of protein extracted from leaves of the stock plants and the variants indicated no difference between them and did not reflect the variation in phenotype.

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The induction of variants through tissue culture has been the main interest for breeders, leading to many studies and reports (Evans and Sharp, 1988; Paek et al., 1993). Plants regenerated from tissue culture can be genetically unstable and have been studied with their karyotype or morphological variations. In addition, a number of studies attempting to increase the somaclonal variant induction frequencies in vitro have been conducted to substitute for conventional breeding methods (Evans and Bravo, 1986). Shoot tip culture is still being used for the propagation of floral and ornamental crops like orchids, lilies and foliage plants because it produces few variants (Debergh and Read, 1991).

When the culture material is not meristem but rather tissue or callus, the regenerated plants generally show a number of variations not inherited in the progeny, which can be attributed to environmental variation. In some cases, however, the variants from tissue culture can be inherited, showing unique morphological characteristics that can not be obtained from cross breeding. Such variants are considered highly valuable in breeding and have been established as cultivars in some floral crops (Orton, 1986; Scowcroft and Larkin, 1982).

In general, the variants selected in vitro have differences, in contrast to those selected from the field, in flower type and color, petal and foliage shape, plant height, flowering days, pollen fertility and activity of particular enzymes. Such variants have been obtained from chrysanthemum (*Dendranthema ×grandiflorum* Kitam.) (Sutter and Langhans, 1981); temperate cymbidiums such as *Cymbidium kanran* Makino, *C. goeringii* Reichb. F., and *C. sinense* Willd. (Paek et al., 1990); african violet (Bhaskaran et al., 1983); and strawberry (*Fragaria ×ananassa* Duchesne) (Schwartz et al., 1981).

The purpose of this study was to determine the variation frequencies of the variants in african violet (*Saintpaulia ionantha* Wendl.) 'Crimson Frost' cultured in vitro.

Materials and methods

Saintpaulia ionantha 'Crimson Frost' was used for the experiment. It has double or semidouble cherry pink petals edged with a clear white line. Leaf surface is partly albino and the blade is nearly orbicular to ovate. The stock plants were grown in a covered greenhouse with subirrigation. Fully expanded young leaves were washed with tap water followed by vacuum sterilization for 10 min in a 1.0% sodium hypochlorite solution containing 0.1% Tween-20. The leaves were rinsed three times with sterile distilled water and median portions of the leaf were cut into 0.5 × 0.5-cm (0.2 × 0.2-inch) segments. The leaf segments were placed into 100 mL Erlenmeyer flasks containing 20 mL of agar medium. The basal medium consisted of MS major salts and Fe-EDTA (Murashige and Skoog, 1962), Ringe and Nitsch microelements with vitamins (Ringe and Nitsch, 1968), and supplemented with 2.0 $mg \cdot L^{-1}$ naphthalene acetic acid (NAA) and 3% (w/v) sucrose. The pH was adjusted to 5.8 with 0.1 N NaOH before the medium was solidified with 0.8% (m/v) bacto agar. The leaf segments were then cultured for 6 weeks to induce multiple shoots at a 25 °C (77 °F) air temperature and a 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity with a 16-h photoperiod provided by cool-white fluorescent lamps.

The multiple shoots induced were propagated for another six weeks on

Table 1. The frequencies of the variants obtained from the culture of homogenized leaf tissue of 'Crimson Frost' african violet.

Plants regenerated (no.)	Normal plants [no. (%)]	Variants including flowers and leaves [no. (%)]
1670	549 (33)	1121 (67)

Table 2. Leaf colors of the plants regenerated from the culture of homogenized leaf tissue in 'Crimson Frost' african violet after 1-year culture in a greenhouse.

Leaf color	Investigated plants [no. (%)]
Chimera (normal, green and white)	549 (33)
All green	918 (55)
All white	203 (12)
Total	1670 (100)

Table 3. Leaf shapes of the plants regenerated from the culture of homogenized leaf tissue in 'Crimson Frost' african violet after 1-year culture in a greenhouse.

Leaf shape	Plants regenerated [no. (%)]
Normal (ovate with crenate margins)	1350 (81)
Elliptical	236 (14)
Undulated margins	84 (5)
Total	1670 (100)

the same medium, after which 5 g leaves with petioles were put into 80 mL of MS liquid basal medium and homogenized for 10 s with a Micromini food processor (model 345; Hamilton Beach Co, St., Joseph, Mich.). One milliliter of homogenate was mixed in a 10-cm-diameter petri dish with 30 mL MS liquid basal medium supplemented with 0.3 mg·L^{-1} NAA, 5.0 mg·L^{-1} kinetin, and 30 g·L^{-1} sucrose. Number of plantlets was calculated at 2-week intervals with five replications. Excised plantlets were then on to MS basal medium supplemented with 2 g·L^{-1} tryptone and 3 g·L^{-1} hyponex fertilizer (6.5N–4.5P–19K) without growth regulators for root induction. When reaching 2 to 3 cm in height, the plants were transplanted into 10-cm-diameter pots filled, by volume, with 40% leaf mould: 30% peatmoss: 30% perlite and grown under greenhouse conditions for one year. Variants were selected from 1670 plants by characterizing their morphology via flowering type and foliage chimeras.

Protein analysis of the variants was carried out to determine the differences between the stock plants and the in vitro-induced variants. Fifty milligram samples of fresh leaves were taken from the stock plants and eight different variants. Samples were ground in 400 mL of extraction buffer (2.5 mL 0.5 M Tris·HCl pH 6.8, 28.5 mL DI H_2O, 6 mL 10% SDS, and 1.0 mL β-mercaptoethanol) over ice. Then the extracts were centrifuged for 20 min at 11,000 g_n. Five microliters of supernatant was subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-page, 0.75 mm discontinuous slab gel, 0.1% SDS) by the method of Laemmli (1970).

Results and discussion

Among the 1670 shoots induced from the culture of homogenized cell clusters, a higher rate of variants in contrast to normal plants was obtained: 1121 variants (67%) and 549 normal plants (33%) (Table 1). The high frequencies of variants in this experiment were in contrast to the results of Molgaard et al. (1991), which showed 0 to 4% variation from the same species.

The number of regenerated plants with variation in leaf color (all green and all white) was 1121 (67%), while the number of those with normal color (chimera) was 549 (33%) (Table 2). Smith and Norris (1983) also reported variations in leaf color ranged from white to green in tissue culture of three variegated leaf chimeras of *Saintpaulia ionantha* Wendl. although there were differences in variation frequencies among species. More plants exhibited a normal leaf shape (81%) than a variant leaf shape (19%) (Table 3). Among the variants, more leaves with oval and acute shapes were observed than those with a curled edge (Fig. 1).

Two types of flower variants were present, and they were more semidouble or double types than single types. Both flower types showed much higher rates of normal color (84% and 64%, respectively) than pink, red or white (Table 4). Few differences in flower colors were observed between semidouble/double type flowers and single type flowers.

A variety of flower patterns and colors were observed (Fig. 2). The colors of the petals ranged from red to white and pale pink to red petals edged with clear white lines. There have been some reports that cultivars with chimeral leaves are stable during culture periods, while the chimeral petals exhibited variation when regenerated (Lineberger and Druckenbrod, 1985; Peary et al., 1988). The leaf segments of the variants cultured in our study showed a number of variations, which could be affected by the chimera layers.

The SDS-PAGE profile of protein extracted from dry leaves of the stock plants and the variants indicated no difference between the stock plants and the variants (Fig. 3).

We propagated the variant plants by leaf cuttings and observed that the propagated plants were different from each other. These results suggest that the variation can originate from the cells whether they are genetically identical or not. It is well known that vegetative propagation produces a mirror image of the parent plant. On the other hand, somaclonal variation appears when plants are produced from single cells as in the case of african violet. Most variations in african violets occur when roots are induced from the leaves of a specific cultivar. A variant can be developed that differs in some noticeable way from its parent. This variant plant can then be propagated further, with set further variants being formed. Some of the cultivars revert back to the original form. The variants, therefore, are by no means

Fig. 1. Typical variant plants obtained from the homogenized leaf tissue of 'Crimson Frost' african violet. (A) Normal type plant (ovate leaf with crenate margins), (B) undulated margined variant with white flower, (C) normal type plant with single pink flower, (D) white-color leaf with crenate margins and acute apices.

Fig. 2. Different types of flowers and flower colors of the variants obtained from the homogenized leaf tissue of 'Crimson Frost' african violet. (A) Normal semidouble pink flower edged with a clear white line, (B) semidouble red flower edged with white line, (C) standard double flower with undulated margined petals, (D) small double flower with undulated margined petals, (E) standard single flower with mixed color and undulated margined petals, (F) small single flower with pale pink and undulated margined petals, (G) different colors of double flowers.

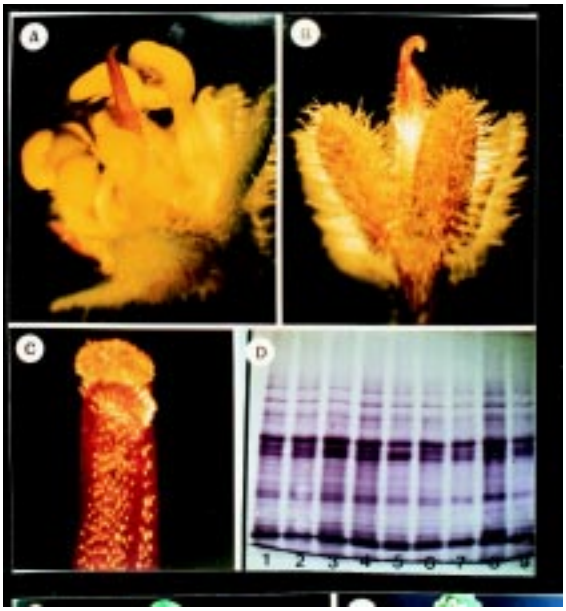

Fig. 3. Different types of flower organs and sodium dodecylsulfate polyacrylamide gel electrophoresis profile of proteins from the variants of 'Crimson Frost' african violet. (A) Nonfunctional stigma and normal anther formation, (B) nonfunctional stigma and aborted anther, (C) normal functional stigma formation; (D) SDS-PAGE profile of proteins extracted from leaves. Lane 1, stock plants; lanes 2–9, variant plants in regards to leaf variegation and flower color.

Table 4. Flower color patterns of the plants regenerated from the culture of homogenized leaf tissue in 'Crimson Frost' african violet after 1-year culture in a greenhouse.

Flower type	Plants observed (no.)	Plants with specified flower color [no. (%)]			
		Mixed (normal)	Pink	Red	White
Semidouble or double	653	549 (84)	39 (6)	26 (4)	39 (6)
Single	99	63 (64)	13 (13)	8 (8)	15 (15)
Total	752	612 (81)	52 (7)	34 (5)	54 (7)

stable. In this study we obtained increased propagation rates of african violet 'Crimson Frost' by homogenized leaf tissue culture as compared to leaf segment culture (Data not shown). And yet, among the propagated plantlets 67% variation was observed, which indicated that homogenized leaf tissue culture was not a proper way to propagate african violet 'Crimson Frost'. When the variant plants were transferred to a greenhouse followed by propagation by leaf cuttings, leaf colors of the propagated plants were segregated to chimera, all green, and all white, indicating genetic instabilities. In this regard, mass propagation of african violet by homogenized leaf tissue culture should be carefully applied since it often results in high frequencies of variant plants. Furthermore, characters of most variant plants were inferior to those of mother plants and therefore, no need to be selected.

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