Effects of Acute Gamma Irradiation on Adventitious Plantlet Regeneration and Mutation from Leaf Cuttings of African Violet (*Saintpaulia ionantha*)

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ABSTRACT

Leaf cuttings of *Saintpaulia ionantha* (African violet) cv. Optima Hawaii, purple flower were acutely irradiated by gamma-rays with the doses of 0, 10, 20, 40, 60, 80 and 100 gray (Gy) to induce mutation. Irradiated leaves (M_1V_0) were planted in plastic trays containing peat moss medium and were placed in a shaded greenhouse. A number of adventitious plantlets per irradiated leaf (M_1V_1/M_1V_0) were recorded. M_1V_1 plantlets were transplanted and were grown to flowering according to the standard African violet culture. The characters investigated in M_1V_1 plants were the number of leaves per plant, plant canopy width, the number of inflorescences per plant, the number of flowers per inflorescence, flower size and mutated characters. The results showed $LD_{50(60)}$ to be 56 Gy. The number of plantlets per leaf decreased as radiation doses increased. No survived leaves were observed at doses higher than 80 Gy. In M_1V_1 generation, averaged width of plant canopy and the number of flowers per inflorescence were not significantly different whereas the number of inflorescences per M_1V_1 plant and flower size were significantly different. Mutation characters observed were – changes in leaf colour, leaf margin, leaf thickness, flower colour, flower size, flower form and plant type. Mutation rate of M_1V_1 plants increased as radiation doses increased. Seven mutants with desirable characters were selected.

Key words: African violet, *Saintpaulia ionantha*, mutation, gamma-rays, acute irradiation

INTRODUCTION

Saintpaulia ionantha is capable of forming new plants from leaf cuttings in a period of six to eight weeks. Cuttings may include the entire leaf, the laminar or a portion of the laminar. Roots are produced endogenously from the thin-walled cells lying between the leaf traces. Shoots originate exogenously in the cells of the epidermis of either the petiole or laminar. Adventitious bud technique together with radiation mutation has been effectively used as breeding method to develop new cultivars for horticultural crops. Adventitious shoot formation in many plants is thought to occur from a single original cell (Broertjes and Keen, 1980). However, many reports involving the induction of mutation suggest that a multicellular origin can occur at low frequency. Sparrow *et al*. (1960) observed that only 1 of 154 plants (0.7%) identified as mutants

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regenerated from irradiated leaf cuttings of African violet was chimera. Colchicine treatment to petioles of *Saintpaulia* leaf cuttings produced one chimeral plant of 29 (3.5%) showing polyploidy (Arisumi and Frazier, 1968). *Saintpaulia ionantha* cv. Utrecht has been used to study the effect of various mutagenic agents, both radiation and chemical, upon mutation frequency and spectrum. In the experiment, a very pronounced dose-rate effect with X - and gamma - rays as well as with fast neutron was observed (Naylor and Johnson, 1937).

Broertjes (1972) studied the effects of acute, chronic or fractionated doses of X-rays or fast neutrons on leaves of *Saintpaulia*. He reported that under certain conditions and with certain treatments, a pre-treatment with 500 rad X-rays and a second dose of 6 krad separated by an 8-h interval, more mutants per 100 irradiated leaves were produced than was the case with the optimum acute dose (3 krad X-rays).

The objective of the experiment was to induce mutation of *Saintpaulia ionantha* cv. Optima Hawaii using gamma-rays as a mutagen.

MATERIALS AND METHODS

Leaf cuttings of African violet (*Saintpaulia ionantha*) cv. Optima Hawaii, purple flower (Violet Group # 89B according to RHS Colour Chart) were acutely irradiated by gamma-rays with a series of doses ranging from 10 to 100 gray (Gy) using Mark I Gamma Irradiator with cesium 137 as the gamma radiation source. Completely randomized design (CRD) with 3 replicates was employed. After irradiation, irradiated leaves (M_1V_0) and the controls (M_0V_0) were planted in the plastic pots containing peat moss. The numbers of survived leaves were recorded 60 days after irradiation to determine LD_{50}. Ninety days after irradiation, a number of adventitious plantlets (M_1V_1) was counted and a number of adventitious plantlets per leaf (M_1V_1/M_1V_0) was calculated. All plantlets were transplanted to the new pots. Their morphological characters were investigated. The numbers of mutant (M_1V_1) with desired traits were selected and were multiplied using leaf cuttings to obtain M_1V_2 plants. Mutants were compared among one another including the control and the desirable mutants would be registered and released as new cultivars.

RESULTS AND DISCUSSION

Irradiation of African violet leaf cuttings with gamma-rays from 10 to 100 Gy showed that the increasing radiation doses resulted in decreasing survival of leaves (Table 1). The results from ANOVA indicated that there was significant difference between the control and the irradiated treatments. The 50% lethal dose of leaves 60 days after irradiation ($LD_{50(60)}$) was estimated to be 56 Gy (Figure 1).Treatments with radiation doses higher than 80 Gy caused all leaves dead. The result agreed with the report of Broertjes (1968) that the survival of African violet leaf cuttings rapidly dropped after acute irradiation with X-rays.

As for the number of adventitious plantlets per leaf, there was significant difference between the control and irradiated treatments (Table 1). The number of plantlets per leaf decreased as radiation doses increased.

The results regarding leaf survival and the number of plantlets per leaf from this experiment and the one carried out by Zhou *et al.* (2006) were found to be varied depending on African violet cultivars, radiation types and doses.

The effects of radiation on growth of the M_1V_1 plantlets are summarized in Table 2. Morphological variability of plantlets regenerated from irradiated leaves was noticed. The characters observed were malformed leaves and flowers, changes in flower colour, size and form (Figure 2), changes in leaf colour, margin, thickness, shape and size (Figure 3). The variability of each character increased with increasing doses. Some characters mutated more frequently than the others, for example, flower colour (Table 3). The numbers of mutant and mutation frequency of plantlets are shown in Table 4. Mutation frequency ranged from 0 – 18.33% in the control and irradiated treatments. The same results were also reported by Broertjes (1969), Okamura *et al*. (2003) and Zhou *et al*. (2006).

Twenty three mutants obtained from this experiment were all solid uniform and reproduced true to true type by leaf cuttings. The result

Table 1 The number of irradiated leaves, the number of survived leaves, and averaged number of plantlets per leaf of African violet after acute irradiation of leaf cuttings with different doses of gamma-rays.

Radiation dose (Gy)	No. of irradiated leaves	No. of survived leaves	Leaf survival (as % of control)	Ave. no of plantlets per leaf
0	45	45a[1]	100a	10.2a
10	45	39b	87b	7.4b
20	45	31c	67c	6.7b
40	45	28c	60c	6.8b
60	45	21d	47d	6.0b
80	45	0e	0 e	0c
100	45	0e	0 e	0c
F-test		*	*	*
C.V. (%)		14.24	14.38	23.12

* significant at 5% level

1 data within columns, means followed by a common letter are not significantly different at the 5% level by DMRT

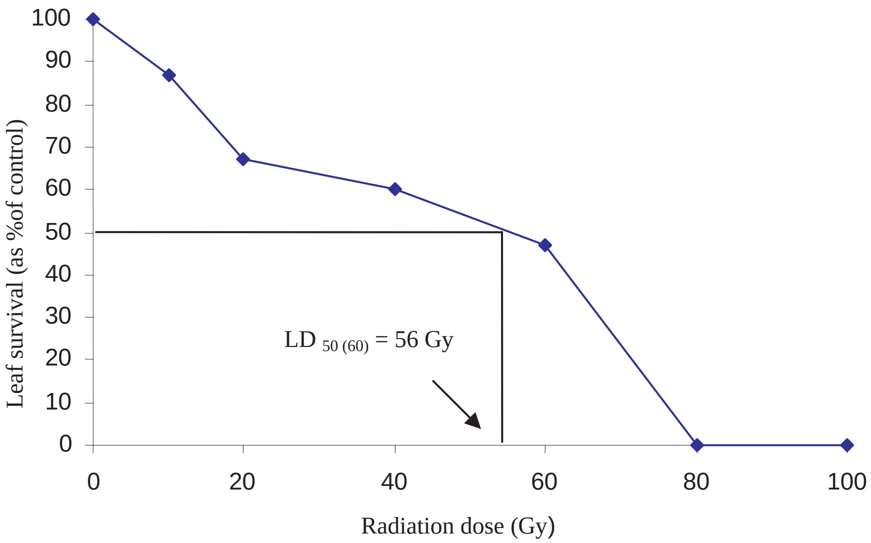

Figure 1 Relationship between radiation dose and survival of African violet leaf cuttings 60 days after acute irradiation with gamma-rays.

Figure 2 Flower colour variability of *Saintpaulia ionantha* cv. Optima Hawaii after treatment of leaf cuttings with different doses of gamma-rays.

Figure 3 Variability of leaf and plant canopy of *Saintpaulia ionantha* cv. Optima Hawaii after treatment of leaf cuttings with different doses of gamma-rays.

Figure 4 *Saintpaulia ionantha* cv. Optima Hawaii and its mutants induced by gamma-rays.

Figure 5 Flowers of the original *Saintpaulia ionantha* cv. Optima Hawaii compared with its mutants.

indicated that the adventitious plantlets, formed on leaf cuttings of African violet, were originated from single cell which supported the previous experiments of Broertjes (1969), Broertjes and van Harten (1978), Broertjes and Keen (1980) and Smith and Norris (1983), Lineberger and Druckenbrod (1985). Naylor and Johnson (1937) indicated that adventitious shoots derived from one epidermal cell, though they also stated that adjacent epidermal cells and parenchyma cells within the petiole contributed to the shoot formation. These authors stated that in

Table 2 Averaged plant canopy width, the number of leaves per plant, the number of inflorescences per plant, the number of flowers per inflorescence and flower diameter of African violet plantlets (M_1V_1) 5 months after transplanting.

Radiation dose (Gy)	Plant canopy width (cm)	No. of leaves per plant	No. of inflorescences per plant	No. of flowers per inflorescence	Flower diameter (cm)
0	21.38	20.50b[1]	4.63b	5.17	2.45a
10	19.27	22.95ab	4.95b	4.51	2.26b
20	19.92	23.58a	5.73a	4.96	2.50a
40	18.76	24.20a	4.55b	4.54	2.34ab
60	20.30	24.87a	4.82b	4.83	2.23b
F-test	ns	*	*	ns	*
C.V. (%)	7.94	5.8	8.07	7.67	3.82

ns non-significant
* significant at 5% level
[1] data within columns, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 3 Mutation spectrum of African violet (*Saintpaulia ionantha* cv. Optima Hawaii) after treatment of leaf cuttings with different doses of gamma-rays.

Mutation characters	Number of mutation	Number of mutation in % of the total
Leaves		
Variegated	1	1.7
Dentate	4	6.9
Thicker	4	6.9
Darker colour	6	10.3
Flower size and form		
Larger	4	6.9
Smaller	5	8.6
Double	2	3.5
Flower colour		
White	12	20.7
Light blue	8	13.8
Pink	1	1.7
Dark violet	11	19.0
Total	58	

conventional propagation from both the petiole and leaf laminar tissue, adventitious shoots originated in epidermal cells. However, many reports involving the induction of mutation suggested that a multicellular origin could occur at low frequency (Sparrow *et al*., 1960; Arisumi and Frazier, 1968). Norris *et al*. (1983) claimed that adventitious shoots of *Saintpaulia* produced *in vitro* were of multicellular origin and that all layers of leaf tissue were involved in adventitious bud formation.

All 23 mutants were propagated by leaf cuttings for further examination of their commercial value. After propagation, seven mutants with desirable traits were selected with the future plan to be registered and released as new cultivars. The remarkable characteristics of each mutant compared to the original cultivar are shown in Table 5, Figures 4 and 5.

Table 4 The total number of M_1V_1 plantlets, the number of mutants and mutation frequency of African violet produced from leaf cuttings acutely irradiated with different doses of gamma-rays.

Radiation dose (Gy)	Total no. of plantlets observed	No. of mutants	Mutation frequency (%)
0	60	0	0.00
10	60	3	5.00
20	60	2	3.33
40	60	7	11.67
60	60	11	18.33
Total	300	23	-

Table 5 Characteristics of seven African violet mutants induced by gamma-rays compared with Optima Hawaii, the original cultivar.

Mutant/control	Radiation dose (Gy)	Plant canopy	Leaves	Flowers
Optima Hawaii (original cultivar)	-	Large (~ 22 cm. in diameter), form irregular	Orbicular, relatively thin, petiole long, margin entire, colour green (Green Group # 137C)[1]	Perfect, large (~2.4 cm. in diameter), 5-6 flowers per inflorescence, 4-5 inflorescences per plant, colour violet (Violet Group # 89B)
Mutant 1	10	Relatively small (~19-20 cm. in diameter), form round	Ovate, relatively small, margin entire, colour green (Green Group # 137C)	Perfect, relatively large (~1.6 cm. in diameter),5-6 flowers per inflorescence, 5-6 inflorescences per plant, colour bluish violet (Violet Group # 87A)
Mutant 2	40	Large (~22-23 cm. in diameter), form round	Orbicular,relatively thick, petiole relatively long, margin entire, colour green (Green Group # 137C)	Perfect : imperfect 7:3, relatively large (~1.7 cm. in diameter), 5-6 flowers per inflorescence, 6-7 inflorescences per plant, colour light violet blue with white (Violet Group # 88C)

Table 5 (cont.)

Mutant/control	Radiation dose (Gy)	Plant canopy	Leaves	Flowers
Mutant 3	40	Relatively large (~21-22 cm. in diameter), form round	Orbicular, relatively small and thick, incurving, petiole relatively long, margin entire, colour green (Green Group # 138A)	Perfect : imperfect 1:4, small (~1 cm. in diameter), 5-6 flowers per inflorescence, 6-7 inflorescences per plant, colour light violet (Violet Group # 88D)
Mutant 4	60	Relatively large (~20-21 cm. in diameter), form round and compact	Orbicular, petiole relatively short, margin dentate, colour green (Green Group # 137A)	Perfect, relatively small (~1.4 cm. in diameter), 3-4 flowers per inflorescence, 4-5 inflorescences per plant, colour white with light violet (Violet Group # 89C)
Mutant 5	60	Small (~17-18 cm. in diameter), form irregular	Orbicular, relatively small and incurving, petiole relatively long, margin dentate, colour green (Green Group #143A)	Perfect : imperfect 3:17, relatively small (~1.3 cm. in diameter), 2-3 flowers per inflorescence, 3-4 inflorescences per plant, colour violet (Violet Group # 87B)
Mutant 6	60	Small (~16-17cm. in diameter), form irregular	Orbicular, margin dentate, colour green (Green Group # 143C)	Perfect : imperfect 1:9, relatively small (~1.2 cm. in diameter), 2-3 flowers per inflorescence, 3-4 inflorescences per plant, colour white
Mutant 7	40	Small (~12-13 cm. in diameter), form round and compact	Ovate, relatively small, incurving, margin entire, colour green with splashed white and grey	Imperfect, few flowers, colour violet (Violet Group # 89B)

[1] Followed the Royal Horticultural Society (RHS) Colour Chart

CONCLUSIONS

The results in irradiation of *Saintpaulia ionantha* cv. Optima Hawaii leaf cuttings with different doses of gamma-rays showed $LD_{50(60)}$ to be 56 Gy. Variations in morphological characters of M_1V_1 plantlets were observed. Seven mutants with desirable traits were selected.

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LITERATURE CITED

Arisumi, T. and L.C. Frazier. 1968. Cytological and morphological evidence for the single-cell origin of vegetatively propagated shoots in thirteen species of *Saintpaulia* treated with colchicines. **Proc. Am. Soc. Hortic. Sci.** 93 : 679 – 685.

Broertjes, C. 1968. Dose rate effects in *Saintpaulia*. pp. 63 – 71. *In* **Mutations in Plant Breeding. II.** (Proc. Panel Vienna, 1967), IAEA.

Broertjes, C. 1969. Mutation breeding of *Streptocarpus*. **Euphytica** 18 : 333 – 339.

Broertjes, C. 1972. Use in plant breeding of acute, chronic or fractionated doses of X-rays or fast neutrons as illustrated with leaves of *Saintpaulia*. Thesis, Centr. Agric. Publ. Doc., Wageningen. **Agric. Res. Rep.** 776, 74 pp.

Broertjes, C. and A. Keen. 1980. Adventitious shoots: Do they develop from one cell?. **Euphytica** 29 : 73 – 87.

Broetjes, C. and A.M. van Harten. 1978. Ornamental crops. pp. 75-193. *In* C. Broetjes and A.M. van Harten (eds.). **Application of Mutation Breeding Methods in the Improvement of Vegetatively Propagated Crops.** An interpretive literature review. Elsevier Scientific Publishing Company.

Lineberger, R.D. and M. Druckenbrod. 1985. Chimeral nature of the pinwheel flowering African violets (*Saintpaulia*, Gesneriaceae). **Amer. J. Bot.** 72(8): 1204-1212.

Naylor, E.E. and B. Johnson. 1937. A histological study of vegetative reproduction in *Saintpaulia ionantha*. **Am. J. Bot.** 24(10): 673 – 678.

Norris, R. E., R.H. Smith and K.C. Vaughn. 1983. Plant chimeras used to establish de novo origin of shoots. **Science** 220 : 75-76.

Okamura, M., N. Yasuno, M. Ohtsuka, A. Tanaka, N. Shikazono and Y. Hase. 2003. Wide variety of flower-color and – shape mutants regenerated from leaf cultures irradiated with ion beams. **Nucl. Instrum. Methods Phys. Res. Sect. B: Beam Interact. Mater. Atoms.** 206: 574 – 578.

Smith, R.H. and R.E. Norris. 1983. *In vitro* propagation of African violet chimeras. **HortSci.** 18(4): 436-437.

Sparrow, A.H., R.C. Sparrow and L.A. Schairer. 1960. The use of X-rays to induce somatic mutations in *Saintpaulia*. **Afric. Violet Mag.** 13(4): 32 – 37.

Zhou, L.B., W.J. Li, S. Ma, X.C. Dong, L.X. Yu, Q. Li, G.M. Zhou and Q.X. Gao. 2006. Effects of ion beam irradiated on adventitious shoot regeneration from *in vitro* leaf explants of *Saintpaulia ionantha*. **Nucl. Instrum. Methods Phys. Res. Sect. B: Beam Interact. Mater. Atoms.** 244: 349 – 353.