Tropical Agriculture Research Vol 16: 253-270 (2004)

New Phenotypes of *Crossandra infundibuliformis* var. Danica through *In-Vitro* Culture and Induced Mutations

W.D.C.J. Hewawasam D.C. Bandara[1] and W.M. Aberathne[2]

Post Graduate Institute of Agriculture
University of Peradeniya
Perdeniya, Sri Lanka

ABSTRACT. *In-vitro shoots of Crossandra infundibuliformis var. Danica were exposed to different doses of gamma rays and colchicines, respectively. Increasing dose of both mutagens caused reduction in mean shoot length at 2 months after treatments in the multiplication medium. Increasing dose of colchicine showed increase in the average number of shoots produced per culture. Shoot tip cultures treated with higher doses (>0.03%) of colchicine were abnormal due to stunted growth at 2 months after treatment in the same medium. At the same time, many leaf abnormalities such as changes in leaf size, shape, margin and apex were also observed in the treated shoots growing under in-vitro conditions. MS medium supplemented with 2mg/l IBA gave better results for in-vitro rooting in treated shoots. However, rootability of these mutants also decreased with increasing treatment dosage. The plants, which withstood both the mutagenic effect and the environmental adversities for three months under net house conditions, survived to flower. At flowering, among the treated population, a single individual from the 3 Krad gamma radiation treatment produced a solid mutant, named "Savindi", with altered leaf shape and flower colour. The plants maintained the new phenotypic characters even after five cycles of vegetative propagation indicating the potential to develop as a novel ornamental product.*

INTRODUCTION

The plant *Crossandra infundibuliformis* var. Danica, originally from India and Sri Lanka is a beautiful flowering shrub that has been introduced to the international floriculture market. It is a natural chimera (spontaneous mutation) of a tropical flowering shrub known as *C. infundibuliformis* L. which belongs to the family Acanthaceae. The challenge today is to develop this plant for its ornamental value, while maintaining existing characters for the export market (Hewawasam *et al*., 2001). To fulfill this target, genetic variability of this crop must be increased.

Use of radiation or chemical mutagens has induced heritable variation to supplement the existing germplasm available for selection, hybridization and clonal propagation (Novak, 1990). Mutation breeding combined with tissue culture has made a significant contribution to plant breeding by introducing new techniques for the propagation and induction of genetic variation. These techniques accelerate breeding pogrammes (Brojects *et al.*, 1978).

---

1 Faculty of Agriculture, University of Peradeniya, Sri Lanka

2 Rice Research Institute, Bathelegoda

Tissue culture techniques that ensure genetic stability (eg: shoot tip and nodal cultures) are particularly useful for *in-vitro* mutation induction and mutant plant regeneration. Direct organogenesis of adventitious buds and /or somatic embrayogenisis on cultured explant offers unique potential to dissolve chimerism and to produce homohistant mutants (Novak, 1990).

Mutant screening under *in-vitro* conditions may eliminate the number of plants from the population that have to be screened further under green house and field conditions (Novak, 1990). *In-vitro* techniques are also convenient for the clonal multiplication of breeding materials prepared for evaluation under different environmental conditions. Plants of the new cultivars especially vegetatively propagated plants and perennials can be rapidly multiplied through *in-vitro* techniques.

Thus, use of mutation breeding and tissue culture techniques on *Crossandra* shoot tips have been initiated with the objectives of finding the potential of gamma radiation and colchicine in combination with *in-vitro* culture for increasing genetic variation in *C. infundibuliformis* var. Danica and to select novel and improved *Crossandra* mutant lines with altered phenotypic characters among the regenarent progenies and utilize them to develop improved varieties/cultivars.

MATERIALS AND METHODS

Location of the experiment

The basic laboratory experiments were carried out in the tissue culture division of Horticultural Crop Research and Development Institute (HORDI), Gannoruwa, Peradeniya and the experiments under net house were carried out in the Research and Development Section, Green Farms Ltd., Marawila during the period from July 1999 to April 2002.

Maintenance of mother plant stock

The mother plants of *Crossandra* "Danica" were maintained as potted plants at the net house with 60% shade. Watering was done sparingly, whenever the soil was dry and this was done by testing the compost with fingers. The plants were watered when compost was light and crumbly. Much care was taken to add only tepid water and excess water was immediately removed to overcome detrimental conditions such as limping and rotting of leaves and base of stems due to over watering.

Culture establishment and treatment with mutagens

Actively growing apical shoot tips of *C. infundibuliformis* var. Danica, collected from 6 months old mother plant stock were excised aseptically and cultured on the medium described by Hewawasam *et al.* (2001).

Treatment with gamma rays

One month after establishment, culture vials (5x6 cm in size) containing 4 shoots (about 3 cm in length) were directly exposed to five different doses (0, 3, 6, 9 and 12 Krad) of gamma rays in a Co_{60} gamma radiation chamber at HORDI. There were 15 culture vials (replicates) per treatment. Explants (shoot tips) were cultured in culture vials that contained 20 ml of culture medium. Treated explants were arranged into 15 culture vials, each with 4 explants (4 replicates per experimental unit) and this was done for each level resulting in a total of 60 culture vials (4 treatment levels x 15 culture vials each) and 240 explants.

Treatment with colchicine

One month after establishment, shoots (about 3 cm in length) were removed from culture vials and treated with 0.03, 0.05 and 0.09% autoclaved aqueous solutions of colchicine by dipping the entire shoot in 150 ml flasks containing different colchicine solutions separately. These flasks were placed in an orbital shaker and allowed to shake gently for 3 h, under aseptic condition. The treated shoots were removed from the containers and rinsed thrice with sterilized distilled water under a laminar floor chamber for removing the traces of colchicine. A similar number of replicates per treatment and the same experimental layout were used as in the experiment done using gamma rays. Equal numbers of cultures were similarly treated with sterilized distilled water, which served as control.

***In- vitro* handling of M_1V_1 generation**

Just after treatment with mutagenic agents, shoot tips were transferred individually to a fresh shoot tip establishment medium and incubated under normal culture environments. Four weeks after culturing, percentage culture survival was recorded and the ED_{50} (effect dose level) for both mutagens were estimated using the Probit Analysis method (Cox and Oakes, 1984). The first vegetative generation in which treatment was performed was referred to as M_1V_1. Similarly the second and subsequent vegetative generations following the treatment were referred to as M_1V_2, M_1V_3.

***In-vitro* handling of M_1V_2 generation**

Individual shoots were dissected from M_1V_1 cultures, and transferred to shoot multiplication medium. Two months after transferring, mean shoot length, average number of shoots per explant and percentage of plants that showed leaf abnormalities were recorded. Percentage of abnormal leaves was calculated according to Koh and Davies, 2000.

$$\text{Abnormal leaves (\%)} = \frac{\text{Treated cultures showed one or more abnormal leaves}}{\text{Total number of treated shoots}} \text{X100}$$

In- vitro handling of M_1V_3 generation

Shoots dissected from M_1V_2 cultures were sub cultured on rooting medium for plantlet regeneration. MS medium supplemented with or without 2mg/l IBA were used as rooting media. Time taken for roots initiation and average number of roots/explant were recorded.

Planting out of regenerated M_1V_3 generation

The mutagen treated and *in-vitro* derived *Crossandra* plantlets were transferred to the laboratory and maintained at room temperature for one week to enhance the acclimatization process. Then they were transferred to plastic pots (12x9 cm) containing a mixture of sterilized garden soil, sand and cow dung (1:1:1v/v) in a plant house and maintained for 3 months. Then they were transferred to 60% shaded net house conditions at Green Farms Ltd.Marawila.

One month after growing under net house these plants were transplanted from 12x9 cm plastic pots to 25x20 cm pots for better growth. Finally, all these plants were carefully maintained by following the standard inter cultural practices including plant protection measures till flowering. At the same time morphological changes were recorded on the basis of visual observations till 5 vegetative generations (M_1V_5) to identify whether the induced morphological characters were stable or not.

Data analysis

All the experiments were layout using Complete Randomized Design (CRD) with 3 replicates. Analyses of Variance (ANOVA) followed by Least Significant Difference Test (LSD) were used to identify the effect of various treatments. Standard Error of the Means (SE) of treatment observations was used to make comparisons and to provide an indication of within treatment variability.

RESULTS AND DISCUSSION

Effect of gamma rays and colchicine in treated *Crossandra* shoot tip cultures under *in-vitro* conditions

In-vitro handling of M_1V_1 generation

Highly negative correlations (r^2=0.99 and 0.98) were observed between treatment dose and survival of cultures (1 month after culture) for gamma radiation and colchicine, respectively (Figs. 1 and 2).

Probit analysis revealed that the ED_{50} values for *in-vitro* derived *Crossandra* shoots were 4.3 Krad of gamma irradiation and 0.04% colchicine, respectively.

In-vitro handling of M_1V_2 generation

When compared with the control, increasing dose of both mutagens caused a reduction in mean shoot length of M_1V_2 shoots cultured in multiplication medium at 2 months in culture (Tables 1 and 2).

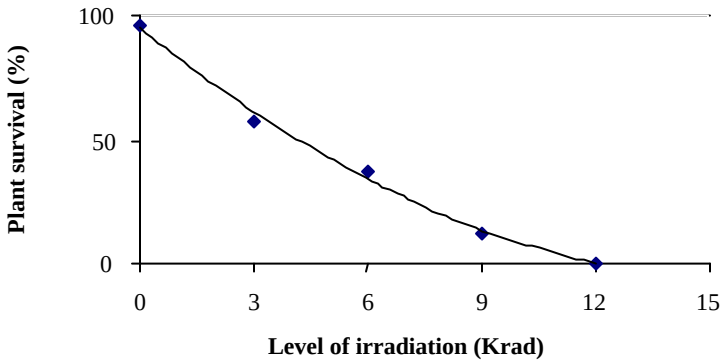

Fig. 1: Effect of Gamma-irradiation on percentage plant survival at 1 month after culturing.

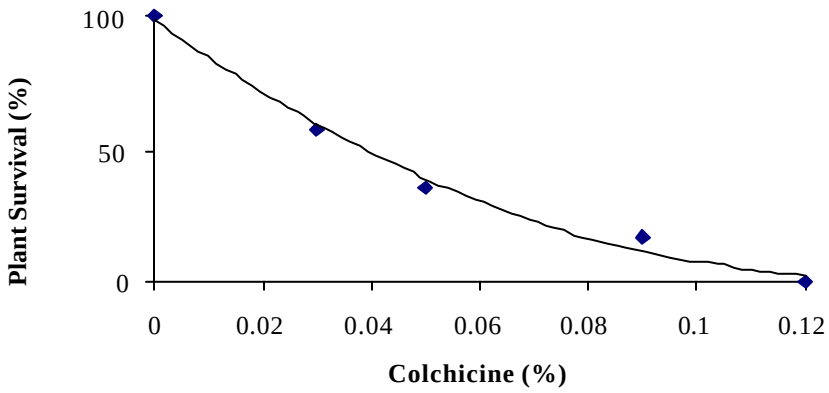

Fig. 2: Effect of colchicine on percentage plant survival at 1 month after culturing.

Reduction in plant height in many tropical ornamental plants after exposure to different mutagenic agents has also been reported by Hoslot (1968) and Datta (1997). Russel and Martin (1952) have reported that physiological effects are responsible for radiation damage as measured by growth reduction.

With increasing treatment dosage of colchicine an increase in mean number of secondary shoots produced per culture was observed (Table 2). However, those shoots were stunted due to reduction of shoot length in culture and therefore phenotypically abnormal at two months in multiplication medium (Plate 1). Datta and Basu (1977) and Datta (1988) have reported from their colchicine treated experiments of *Trichosanthes* and *Chrysanthemum* that the basic cause of abnormal plant growth is associated with non-heritable physiological disturbances and growth substances induced by colchicine. The exact nature of these, however, could not be established.

Table 1. Effect of gamma radiation on mean shoot length and mean number of shoots at 2 months in culture.

Treatment Dose (Krad)	Mean shoot length (cm) ±SE	Mean No. of secondary shoots per culture±SE
0	9.0±0.53 [a]	6.8 ±0.74 [a]
3	5.8±0.56 [b]	5.2 ±0.54 [b]
6	4.0±0.61 [c]	3.6 ± 0.40 [c]
9	2.5±1.17 [d]	3.0 ± 0.28 [d]

SE= Standard Error of Mean, Means within a column with different superscripts are significantly different at p=0.05.

Table 2. Effect of Colchicine on mean shoot length and mean shoot number at 2 months in culture.

Treatment Dose (%)	Mean shoot length (cm)±SE	Mean No. of secondary shoots per culture±SE
0	8.5±0.36 [a]	6.6±1.05 [d]
0.03	4.2±1.78 [b]	11.6±1.61 [c]
0.05	2.7±0.97 [c]	14.6±2.68 [b]
0.09	2.5±0.60 [c]	18.5±1.95 [a]

SE=Standard Error, Means within a column with different superscripts are significantly different at p=0.05.

Plate 1. Different growth responses of *Crossandra* exposed to mutagens at 2 month in multiplication medium.

However, increasing dosage of gamma rays showed decrease in mean number of secondary shoots produced per culture (Table 1). In this experiment many leaf abnormalities such as change in leaf size, shape, margin and apex were observed in the treated shoots (M_1V_2) growing in multiplication medium. These leaf abnormalities were common in both gamma rays and colchicine treated populations. There were no mutagen type or dose specific abnormalities. The development of such abnormal leaves in the treated populations was probably due to physiological disturbances and chromosome aberrations (Datta, 1997). The percentage of these abnormal leaves increased with increasing gamma ray dose and colchicine concentration (Figs. 3a and 3b).

***In-vitro* handling of M_1V_3 generation**

At rooting stage, untreated shoots (control) showed better rooting ability giving the highest percentage of rooting and mean number of roots per plant within shortest time period in hormone free MS medium than treated shoots (Table 3). The probable reason for this observation could be the necessary endogenous hormone balance required for rooting in untreated shoots that could have been maintained in the hormone free medium (Hewawasam *et al*., 2001).

When compared with hormone free MS medium, MS medium supplemented with 2 mg/l IBA gave better results for root initiation in treated shoots. However, 3 Krad gamma ray and 0.03% colchicine treated shoots produced highest mean number of roots per plant giving 55 and 53 % rooting, respectively. Increasing dosage of both mutagens caused root development to be postponed giving poor percentage rooting and less number of roots per plant. Instead of rooting, the treatment with 0.09 % colchicine caused unnecessary basal callus development at explant base even 9 weeks after culturing (Table 4). Qin-zhiwei *et al.,* (1995) have also observed an inhibitory effect on root development among regenerated *Brassica oleraceae* L. plants after gamma irradiation.

Destruction and inactivation of exogenous auxin balance by x-irradiation and colchicine have earlier been reported by Skoog (1935) and Gordon and Weber (1953).

That could be the same reason for this inhibitory effect on root development in both gamma irradiated and colchicine treated *Crossandra* plantlets in this experiment.

Growth of gamma rays and colchicine treated *Crossandra* shoot tip cultures under net house conditions

Effect of mutagenic agents on plant survival

When plants were transferred to a plant house condition, the survival rate of regenerated plants dropped further with increased dosage within one month after acclimatization. However, the plants which withstood both the mutagenic effect and the environmental adversities for another three months under normal plant house conditions survived till flowering (Table 5).

Sax (1942) and Lea (1947) have shown that survival of plants to maturity and reduction in seedling height depend on the nature and extent of chromosomal damage. Increasing frequency of chromosomal damage with increasing dose may be responsible for less germinability and reduction in plant survival and plant growth.

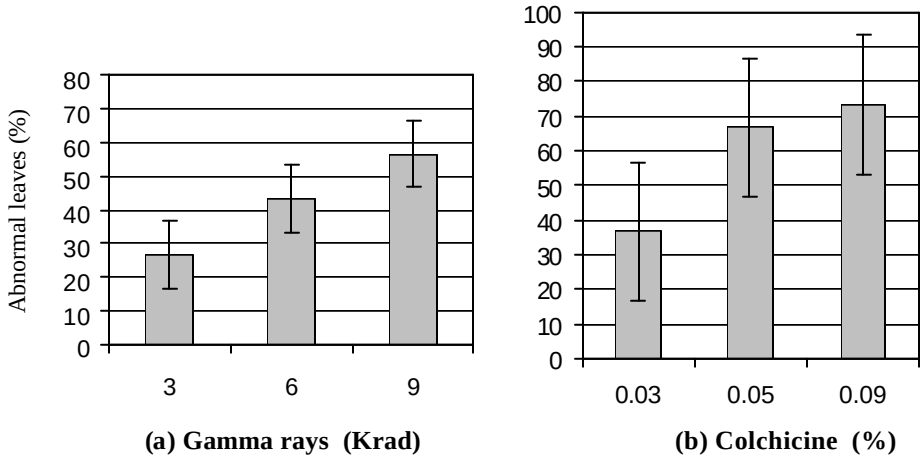

Fig. 3. Percentage shoots which showed abnormal leaves with (a) gamma rays and (b) Colchicine treated cultures at 2 months in culture.

Effect of mutagenic agents on plant height

A reduction in plant height was observed at 3 months after transfer to the normal plant house conditions (Table 6 and Plate 2). Effect of different

mutagenic agents on plant height has been reported by number of researchers and this aspect has been reviewed earlier in detail by Gunckel and Sparrow (1954).

Further, the reduction in plant height after exposure to ionizing radiations has earlier been reported by Datta and Basu (1977). Sparrow and Gunckel (1955) have suggested that chromosomal damage associated with non-chromosomal damage due to radiation effect plays an important role in growth reduction.

Table 3. Effect of different levels of gamma rays and colchicine on *in-vitro* rooting of treated *Crossandra* shoots in MS medium.

Treatment	Levels	Rooting (%)	Mean time (weeks) to initiate roots± SE	Mean number of roots/plt± SE
Gamma	0	100	5.5±0.74 [d]	6.5±1.14 [a]
(Krad)	3	12	8.0±0.75 [c]	3.2±0.55 [b]
	6	80	9.5±0.91 [b]	1.5±0.92 [c]
	9	6	10.5±0.83 [a]	1.3±1.88 [d]
Colchicine	0	98	6.0±0.91 [d]	5.6±1.59 [a]
(%)	0.03	16	8.5±0.91 [c]	4.2±1.07 [b]
	0.05	5	10.0±0.64 [b]	2.3±0.48 [c]
	0.09	2	11.5±0.06 [a]	0.6±0.05 [d]

SE=Standard Error, Means within a column with different superscripts are significantly different at p=0.05

Table 4. Effect of different levels of gamma rays and colchicine on *in-vitro* rooting of treated *Crossandra* shoots in MS+IBA (2mg/l) medium.

Treatment	Levels	Rooting (%)	Mean time (weeks) to initiate roots± SE	Mean number of roots/plt± SE
Gamma	0	94	7.20±1.01 [c]	3.0±0.70 [b]
(Krad)	3	55	7.33±0.61 [c]	5.4±5.4 [a]
	6	20	8.86±8.86 [b]	3.0±3.0 [b]
	9	8	10.00±10.00 [a]	2.1±0.35 [c]
Colchicine	0	90	6.8±0.77 [c]	3.2±0.41 [b]
(%)	0.03	53	6.5±0.51 [c]	4.4±1.20 [a]
	0.05	18	9.0±0.75 [b]	3.6±0.61 [b]
	0.09	0	10.0±0.64 [a]	0.0 ¨

SE=Standard Error, Means within a column with different superscripts are significantly different at p=0.05, ♦= Basel callus development was observed instead of root initiation

Effect of mutagenic agents on leaf/plant abnormalities

There were no abnormal leaves in the control populations. All the treated plants showed some morphological abnormalities in leaves. However, these abnormalities were observed only in the early stages of growth (about 3 weeks to 4 months). There were no mutagen type or dose specific abnormalities. The leaf abnormalities include changes in shape (unequal development of lamina), size, margin and apex of leaves.

The development of forked and double leaves also occurred in treated populations (Plate 3). The developments of such abnormal leaves in the treated populations were probably due to physiological disturbances and chromosomal aberrations. Radiation induced abnormal plant growth has been reported by number of workers and has been variously interpreted (Hewawasam, 2003). It has been suggested that chromosome breakage, reduction in the auxin level, change in enzyme activity and variation in ascorbic acid concentration are some of the factors which lead to the development of abnormal leaves (Datta, 1997).

Effect of mutagenic agents on number of shoots per plant

Decrease in shoot number with increasing treatment dosage was recorded in gamma ray treated plants at 3 months after transferring to the normal plant house conditions.

In colchicine treated plants, increased branch number (bushy habit) was recorded at 0.03% concentration level, which again decreased with increasing treatment dosage (Fig. 4). Raghuvanshi and Singh (1974) reported similar result in *Trigonella foenum-graecum*, which showed a bushy habit from colchicine treated populations.

Table 5. Comparison of survival % of regenerated *Crossandra* plants (M_1V_3) at *in-vitro* rooting stage and under net house conditions (4 months after acclimatization).

Treatment	Level	Survival of rooted plantlets under in-*vitro* conditions (%)	Survival of plants under net house conditions (%)
Gamma (Krad)	0	94	76
	3	55	64
	6	20	40
	9	8	0
Colchicine (%)	0	90	95
	0.03	53	68
	0.05	18	42
	0.09	0	0

Table 6. Effect of different doses of gamma rays and colchicine concentrations on plant height.

Treatment	Dose /concentration level	Mean plant height (cm ±SE)
Gamma (Krad)	0	20.43± 0.57 [a]
	3	15.70± 0.57 [b]
	6	08.95± 0.51 [c]
Colchicine (%)	0	18.20± 0.63 [a]
	0.03	13.60± 0.66 [b]
	0.05	11.57± 0.63 [c]

SE= Standard Error, Means within a column with different superscripts are significantly different at p=0.05

0 Krad **3 Krad** **6 Krad**

Plate 2. Effect of different doses of Gamma rays on plant height of *in-vitro* derived *Crossandra* plantlets growing under normal plant house conditions.

Effect of mutagenic agents on flowering behavior

For this study, the number of months from planting to full bloom was taken in to consideration. All the treated plants of different exposures showed delay in full blooming as compared to the respective controls (Fig. 5). This may be due to the reduction in the rate of various physiological processes, which decreased after exposure to different muragenic treatments. The exact nature of effect on flowering time is not fully understood. However, radiation induced earliness and lateness in mean flowering time have been reported by Gupta and Datta, (1978). However, in this study, there was no significant difference between gamma ray and colchicine treated plants in the time taken to full blooming when compared with the controls (Fig. 5).

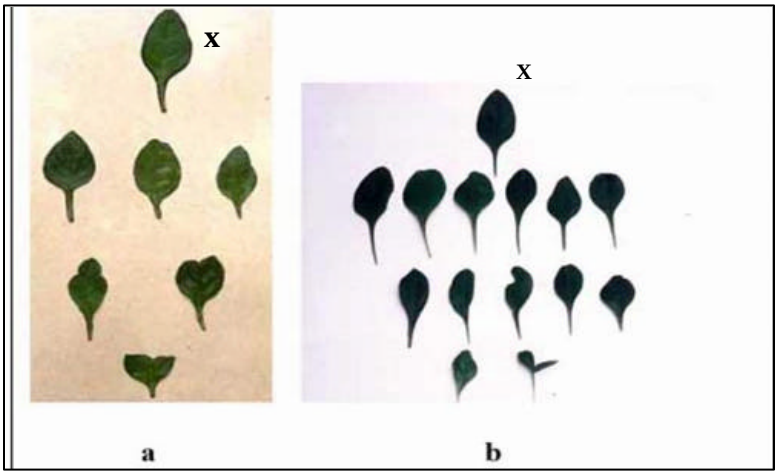

Plate 3. The leaf abnormalities observed in plants growing under normal plant house condition at 3 months after transplanting (a: Colchicine induced leaf abnormalities; b: Gamma ray induced leaf abnormalities; x – Represent leaf from control treatment (normal leaf).

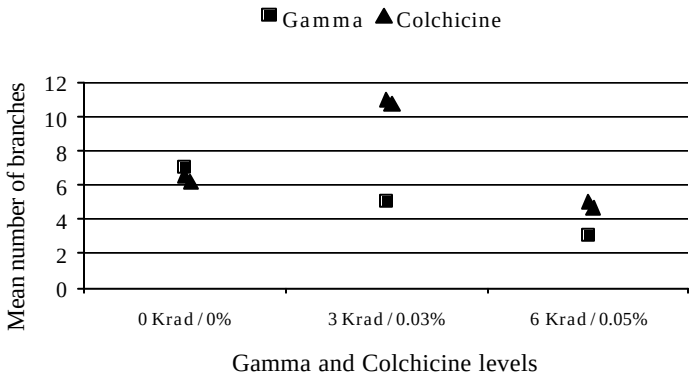

Fig. 4. Effect of Gamma rays and colchicine on number of branches of in-*vitro* derived *Crossandra* plants.

Induced somatic mutations by gamma irradiation and colchicine treatment

There were no changes in the original characters in *in-vitro* derived untreated (the control) plants. Among gamma ray treated populations, a single individual from the 3 Krad treatment (Table 7) showed altered leaf shape from wide to narrow and flower colour from orange to pink (Plate 4 and Table 9). The 5 plants treated with 0.03% and 2 plants treated 0.05% colchicines also showed some bigger and malformed flowers compared to control (Table 7).

Similar results have also been reported in other ornamental plants such as *Chrysanthemum*, Rose (Datta, 1997), and *Tulip* (Van Eijk *et al.*, 1987). Broertjes and Keen (1980) have isolated a number of stable/solid mutants in *Chrysanthemum* cv.'Horim' by successive use of radiation-induced mutants in their mutation breeding programme.

The radiation induced flower colour changes may be due to chromosomal aberrations, changes in chromosome number, gene mutation, rearrangement of different histogenic layers and mutation occurring in the biochemical pathway leading to pigment formation (Heslot, 1968; Ichikawa *et al.*, 1970).

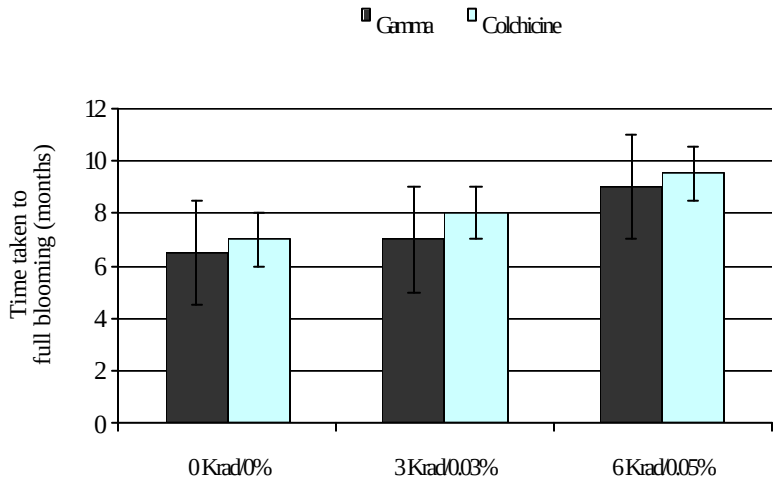

Fig. 5. Effect of gamma rays and colchicine on flowering behavior of in-vitro derived *Crossandra* plants.

On the basis of these interpretations the exact mechanism of induction of somatic mutations cannot be explained with certainty. Therefore, much attention should be paid in future studies for the comparative analysis of original *Crossandra* cultivar and their respective induced mutants on anatomical, palynological, cytomorphological, radiosensitivity and biochemical characters for better and clear understanding of the

exact mechanism involved in the origin and evolution of somatic flower colour mutations at molecular level (Datta, 1997).

Table 7: The mutation rate/frequency of having solid mutants in *Crossandra* by 3 Krad gamma ray and 0.03% and 0.05% colchicine induced *in-vitro* mutagenesis.

Mutagenic Agent	Number of treated plantlets which survived in *in-vitro* multiplication and rooting stages			Mutation rate
	M1V1	M1V2	M1V3	
3 Krad Gamma radiation	60	240	960	1/960
0.03% Colchicine	60	195	585	5/585
0.05% Colchicine	60	180	370	2/370
Control (no treatment)	60	300	920	no

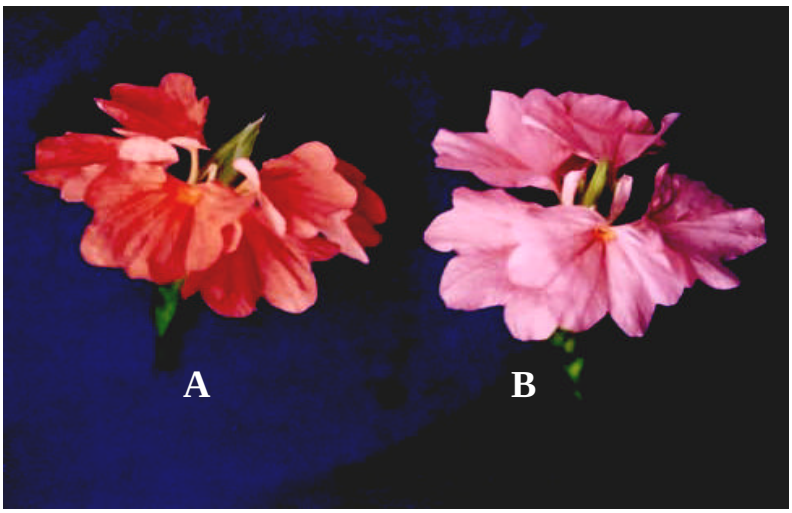

Plate 4: Comparison between normal (A) and mutated (B) flowers of *Crossandra infundibuliformis* var. Danica.

The novel mutant obtained by gamma irradiation in this research could maintain the five generations of vegetative multiplication. (Table 8) and therefore it was identified as a solid (permanent) mutant.

Table 8: The number of vegetative shoots multiplied in each vegetative generation of mutant *Crossandra* "Savindi".

Vegetative generations	Number of shoots in each generation	plants survived till flowering (%)	Stability of phenotypic characters (Yes/No)
V_1	2	100	Yes
V_2	6	66.6	Yes
V_3	16	75	Yes
V_4	38	76.3	Yes
V_5	73	89.0	Yes

Table 9: Characters of "Danica" and its induced mutant "Savindi".

Character	**"Danica"**	**"Savindi"**
Plant height (cm±SE)	23.3± 0.18	18.00± 0.83
Leaf length (cm ± SE)	13.00±1.97	7.67± 2.44
Leaf width (cm ± SE)	5.40± 0.56	4.48 ± 0.24
Leaf shape	spatulate (wide)	oblanceolate (linear)
Flower colour	orange	pink
Flower petal size (cm^2± SE)	7.35 ± 0.73	7.19 ± 1.81
Total flowers/plant	6.00 ± 0.97	6.70 ± 0.67
Length of flowering spike (cm ± SE)	6.00 ± 0.98	9.20 ± 1.20
Petiole length (cm ± SE)	6.25 ± 1.25	3.35 ± 1.32
Time taken to full bloom (months ± SE)	6.35± 0.41	7.50± 0.28

Significant at p=0.05

This solid mutant was labeled as a new cultivar in the name of 'Savindi' and it is now being assessed for its suitability for release as a novel ornamental product to the international floriculture market.

CONCLUSIONS

01. *In-vitro* culture and induced mutations showed promising results for increasing phenotypic variation in *C. infundibuliformis* var. Danica that can be *in-vitro* propagated by apical shoot tips.
02. The procedure of the mutagenesis of C. *infundibuliformis* var. Danica elucidated in this research will be helpful to future researchers who are interested in the mutagenesis of *Crossandra* spp. particularly since this is the first report on the mutagenesis of the C*rossandra* spp.
03. Much attention should be paid in the future studies for the comparative analysis of original *Crossandra* cultivar and their respective induced mutants for better and clear understanding of the origin and evolution of somatic flower colour mutations at molecular level.

ACKNOWLEDGMENTS

Authors wish to thank Dr. S. S. B. D. G. Jayewardene (former Director General of Agriculture) and Dr. C. Kudagamage (Director, HORDI) and Mr.A. Svinningen (Managing Director, Green Farms Ltd.) for their support to conduct the research at HORDI and Green Farms Ltd. The financial assistance provided by the Council for Agricultural Research Policy (CARP) and the technical assistance extended by Mr. Ranga Ekanayaka and Ms. Manel Udewela are also gratefully acknowledged.

REFFERENCES

Broertjes, C. and Van Harten, A.M. (1978). Application of Mutation Breeding Methods in the Improvement of Vegetatively propagated Crops. An Interpretive Literature Review. Elsevier Scientific Publishing Co. Amsterdam.

Broertjes, C. and Keen, A.(1980). Adventitious shoots: do they develop from one cell? Euphytica 29: 73-87.

Broertjes, C and Van Harten, A.M. (1988). Applied Mutation Breeding for Vegetatively Propagated Crops. Elsevier Science Publishers. Amsterdam.

Cox, D.R. and Oakes, D. (1984). Analysis of Survival Data. Champman and Hall, London.

Datta, S.K. and Basu, R.K. (1977). Abnormal Plant Growth in M_1 and C_1 gene ratios of two species of *Tricho santhes*. Trans.Bose. Res. Inst. 40 (3): 63-67.

Datta, S.K. (1988). 'Agnisikha' – a new Chrysanthemum cultivar evolved by gamma irradiation. The Chrysanthemum 43 (1): 40.

Datta, S.K. (1997). Ornamental Plants. Role of mutation. Daya Publishing House, Delhi. pp.1-9.

Gordon, S.A. and Weber, R.P. (1953). Enzymatic radio sensitivity of auxin biosynthesis. Abst. No.21. Radiation Research Soc, Iowa city, June 22-24.

Gupta, M.N. and Datta, S.K. (1978). Effects of gamma rays on growth, flowering behavior and induction of somatic mutations in *Chrysanthemum.* SABROJ 10 (2): 149-161.

Hewawasam, W.D.C.J., Bandara, D.C., Abeyarathne, W.M. (2001). *In–vitro* Propagation of *Crossandra infundibuliformis* Var.Danica through shoot tip and callus culture. Tropical Agricultural Research.13: 328-340.

Hewawasam, W.D.C.J. (2003). Creating Novel Phenotypes of *Crossandra* infundibuliformis var. 'Danica' through Tissue Culture and induced Mutations. M.phil. Thesis. PGIA, University of Peradeniya.

Heslot, H. (1968). Mutation research done in 1967 on barley, roses and marigolds. A progress report, Mutation in plant breeding. IAEA, Vienna. pp 153-159.

Ichikawa, S., Yamakawa, K., Sekiguchi, F. and Tatsuno, T. (1970). Variation in somatic chromosome number found in radiation induced mutation in *Chrysanthemum morifolium* Hemsl. cv. yellow Delaware and Delaware. Radiat.Bot. 10: 557-562.

Koh, Y.C.and, Davies, F.T. (Jr). (2000). Mutagenesis and *in-vitro* culture of *Tillandsia fasciculata* Swartz var. *fasciculata* (Bromeliaceae). Scientia Horticulturae.87: 225-240.

Lea, D.E. (1947). Action of radiation on living cells. Cambridge University Press. London.

Mackey, J. (1956). Mutation Breeding in Europe. Brookhaven Symp. Biol. 9: 141-156.

Novak, F.J. (1990). *In-vitro* Mutation System for Crop Improvement. In: IAEA, Proceedings of An International Symposium on the Contribution of Plant Mutation Breeding to Crop Improvement. International Atomic Energy Agency. Vienna. pp 327-340.

Qin-Z, An-Y, Wang-C, Xu-R.X., Gin-Z., An-Y., Wang-C., Xu-R. and Zhu-D.W. (1995). Study on the effect of $C0_{60}$ Gamma-ray on the Callus of Broccoli (*Brassica oleraceae* L.) var.Italica. Acta Horiculturae. 402 (1): 185-193.

Raghuvanshi, S.S. and Singh, A.K. (1974). A possible mutagenic effect of colchicines in *Trigonella foenum-graecum.*Cytologia 39:73-76.

Russel, R.S. and Martin, R.P. 1952. Biological Effects of Radiation Hazards of Atomic Energy. Oxford Univ. Press, London: 18-32.

Sparrow, A.H. and Gunckel, J.E. (1955). The effect on plants of chronic exposure to gamma radiation from radiocobalt. Proc. Intnl. Conf. Peaceful Uses of Atomic Energy, Geneva 12: 52-59.

Sax, K.(1942). The mechanism of x-ray effects on cells. J. Gen. Physiol. 25: 533- 537.

Skoog, F. (1935). The effect of x-irradiation on auxin and plant growth. J. Cells Comp. Physiol. 7: 227-270.

Van Eijk, J.P., Nieuwhof, M., Van Keulen, H.A. and Keijzer, P. (1987). Flower colour analysis in *Tulip (Tulipa* L.). The occurrence of carotenoids and flavonoids in *Tulip* Tepals. Euphytica 36: 855-862.