Tropical Agricultural Research Vol. 19: 265 - 272 (2007)

Improvement of *Gymnostachyum species* by Induced Mutation

T. T. Khaing, A.L.T. Perera[1], V.A. Sumanasinghe[1] and D.S.A. Wijesundara[2]

Postgraduate Institute of Agriculture
University of Peradeniya
Peradeniya, Sri Lanka

ABSTRACT. *The genus Gymnostachyum of family Acanthaceae consists of beautiful flowering shrubs and herbs, native to Sri Lanka. It can be used as an ornamental plant as well as a ground cover plant in landscape designing. The present research programme was initiated to create variations in local Gymnostachyum plants using mutation induction techniques. The materials used in this experiment were from Gymnostachyum zeylanicum. One to two months after establishment of the vegetatively propagated plants, the shoot cuttings were taken in order to subject them to mutations by gamma irradiation and colchicine treatments. There were changes in leaf size and shape in all colchicine treatments. The treated plants had shorter, oval shaped and rounded-based leaves compared to the large, oblong and tapering-based leaves in controls. They also showed early flowering and shortening of the flower stalk to 5 - 7 cm in length compared to the control (30 - 45 cm). All the dosages of gamma treatments resulted in retarded growth while the control plants grew well and produced flowers. These plants showed dark green colored leaves compared to the control.*

INTRODUCTION

Genetic variation is necessary in crop improvement programs. Induced mutations are highly effective to create genetic variations and have successfully assisted in developing improved and new cultivars among both seed and vegetatively propagated crops (Jain, 2006). The frequency of induced mutations is much higher than spontaneous mutations (Rieseberg, 2000). A mutation can lead to loss of function or introduce a new function of a gene (Griffiths, 2000). Induced mutation offers the breeder an approach for improvement of an unknown genetic source, especially vegetatively propagated plants which are cross-pollinated, highly heterogeneous and polyploids (Schum, 2003).

Generally, physical and chemical mutagens are tools for enhancing and generating genetic variation by inducing mutations (Gregory, 1996; Yamaguchi, 2003). Colchicine, a heavily used chemical agent, disrupts mitosis, halting the process at metaphase (Matthew, 1979). It can be used as the most effective tool to create different polyploid populations of plants species. The application of gamma rays and other physical and chemical mutagens in plant breeding in the last seven decades has increased crop biodiversity and productivity in different parts of the world (Muggleston, 1995). Heavily ionizing radiation can result in considerable biological damage. The gama-ray irradiation causes mutation in plant cells, depending upon the frequency and dosages of the treatments (Micke, 1999).

[1] Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka.

[2] Royal Botanic Gardens, Peradeniya, Sri Lanka.

The present status of the utilization of induced mutations in plant breeding shows that with induced mutations, in principle, successes can be expected similar to those with the conventional breeding methods (Micke, 1999). In vegetatively propagated crops, induced mutations can improve existing cultivars faster than conventional breeding methods (Griffiths, 2000). Induced mutations may also be viewed as a complementary tool to conventional breeding (Broertjes and Vanharten, 1978; 1988). According to FAO/IAEA database, of the 465 mutants released among the vegetatively propagated plants, most are floricultural plants and a few are fruit species (Micke, 1999).

Some important ornamental plants, both potted and cut flowers such as Chrysanthemum, Orchids, Pelargonium, Canna, Carnation, Alstroemeria, Dahlia, Achimenes, Begonia, Streptocarpus, Azalea, Bougainvillea and Rose were improved by mutation induction techniques (Ahloowalia and Maluszynski, 2001; Okamura, 2003). *Gymnostachyum* species have beautiful flowers of different colors (yellow- blue- purple). They can be used for indoor and outdoor landscape designing and as a ground cover plant. However, there has not been any breeding work carried out to enhance the variability of this plant locally.

Though attractive both in foliage and flowers, Gymnostachyum has yet to be domesticated. Therefore, the main objective of the present research programme was to improve local *G. zeylanicum* as a commercially appealing ornamental plant using mutation breeding technique. The specific objectives were to produce larger flowers, higher number of flowers on a short stalk, changes in type of inflorescence, variation in leaf shape and compact plants.

MATERIALS AND METHODS

Materials

Plant materials of *G. zeylanicum* used in this experiment were obtained from the *ex-situ* collections available at the Royal Botanic Garden (RBG), Peradeniya. The Gymnostachyum plants were multiplied by cuttings and established under a net house to obtain larger number of plants for mutation treatment. The plants were treated with systemic insecticides to control mealy bugs. The plants were treated with the two mutagens, gamma rays and colchicine. One to two months after establishment of the vegetatively propagated plants, the stem cuttings were taken for the mutation treatments. The cuttings were treated with different doses of gamma irradiation and different concentrations of colchicine solutions.

Treatment with gamma rays

The cut stems were directly exposed to five different doses (0 Krad, 3 Krad, 6 Krad, 9 Krad and 12 Krad) of gamma rays in a 60Co gamma radiation chamber at HORDI. There were 3 replicates and each treatment consisted of 30 cut stems. After gamma irradiation, the treated stems were planted in black polythene bags containing 1:1:1 sand: topsoil: cow dung mixture and placed at the RBG, Peradeniya and the Department of Agricultural Biology, University of Peradeniya. The maximum dose of 12 Krad caused early lethal effects to the plants. The treatment of 9 Krad showed better survival of plants. Since all the treated plants did not produce any mutants, another set of fresh plant materials were exposed to four different doses of 0 Krad, 7 Krad, 9 Krad and 11 Krad. It included 25 cut stems in each treatment with three replicates.

Treatment with Colchicine

The shoot cuttings were treated with autoclaved aqueous solutions of colchicine in six different concentrations (0.00%, 0.02%, 0.04%, 0.06%, 0.08% and 0.10%). There were five replicates which included 45 cut stems in each treatment. The entire surfaces of the stem were dipped in 150 mL flasks containing different colchicine solutions separately. The materials were kept for 24 hrs in colchicine solution. After colchicine treatment, the cut stems were planted in black polythene bags containing sand: leaf mold (1:1) mixture and placed in the net house at the RBG, Peradeniya and the Department of Agricultural Biology, University of Peradeniya.

Characters evaluated

The data collected on the following characteristics were analyzed using MINITAB-14, Mtb EXE version.

i. Leaf shape (oblong - oval)
ii. Size of leaf (leaf area- cm^2); product value between the length and width of leaf blade at the longest axis and widest point of the leaf. The data were collected from mature leaves of 10 - 15 individual plants for analysis. In the case of gamma irradiated plants, data were collected two months after gamma irradiation.
iii. Size of flower (cm): the width of open lips of the flower x length of the corolla tube.
iv. Length of stalk (inflorescence): measurement from the basal node of the inflorescence to the top.
v. Number of flowers: Number of flowers per node was measured as an indication of compactness.

RESULTS AND DISCUSSION

The analysis of variance showed significant differences in all treatments of gamma irradiations, indicating the possibility of recovering useful variants in some characters. There was no difference between colchicine treatments and control for leaf area. Colchicine treated plants showed significant differences for plant height and number of leaves.

Treatment with gamma rays

Differences in characteristics of the maximum number of leaves, minimum length of flower stalk and leaf size (by area) were observed in gamma treated plants when compared to the control (Table 1, Figures 1A - 1D).

Table 1. Changes in characters due to gamma irradiation treatment.

	control	3 Krad	6 Krad	9 Krad	12 Krad
Max. no. of leaves (no)	14.0	3.1	1.2	1	0.8
Min. size of leaves (cm^2)	4.9	2.3	2.5	2	1.8
Min. length of inflorescence stalk(cm)	49.65	20.7	28.82	6.44	5.02

Figure 1. Changes in the characteristic features of floral arrangement, inflorescence length and leaf color due to gamma irradiation (7 Krad).

Note: A: Opposite and decussate aestivation on floral axis in normal plant, B: Whirled position of flowering pattern, C: Shortening of flower stalk, D: Dark green leaves.

Two months after gamma irradiation, all the dosages of gamma treatments resulted in poor growth pattern while the control plants were growing well. Figure 1A. shows the shortening of flower stalk in gamma irradiated plants compared to control. In the first two months after irradiation both treated and control plants showed similarity in growth, and vigor. The gamma treated plants showed dark green colored leaves compared to the control (Figure 1B). When gamma dosage changed to 7 Krad, it resulted in the production of flowers with different coloration of flower stalk and different flowering pattern (Figure 1C and D).The upper position of flowers on the inflorescence showed a whirled position in the gamma treated plants while the control plants showed normal opposite and decussate position. The color of inflorescence stalk also changed to reddish brown in the gamma treated plants while the control showed pure green color.

Treatment with Colchicine

There were changes in leaf size and shape in all colchicine treatments (Table 2). The character variation observed due to colchicine treatments are shown in Figures 2A - 2F).

Table 2. **Changes in characters due to Colchicine treatment.**

Colchicine Conc: (%)	Change in leaf shape (oval) (%)	Max. size of flower (cm)	Plants with reddish flower stalk (%)	Minimum flower stalk length (cm)	Compact plants with short flower stalk (%)
Control	-	0.5	-	45	-
0.02	22	0.8	18	30	-
0.04	23	0.9	28	15	8
0.06	16	0.9	26	9	13
0.08	36	1.0	21	7	21
0.1	38	1.0	36	5	18

Table 3. **Comparison of mutants with normal plants.**

Objectives	Gamma 7Krad only	Colchicine	Normal (Control)
Larger flower size	0.7 cm	1.0 cm	0.5 cm
Position of flower on stalk	Whirled position	Whirled with mass flowers	Opposite and decussate with less flowers
Type of inflorescence	Not truly raceme	Not truly raceme	True raceme
Length of inflorescence	7-9 cm (erect)	5-7cm (erect)	45 cm (long and drooping)
No. of flowers per node	4-7	4-7	1-2
Coloration of flower stalk	Reddish	Reddish	Greenish
Shape of leaf	Ovate with short and rounded base	Ovate with short and rounded base	Oblong with attenuated base

Figure2. Changes in plant architecture due to colchicine effects.

Note: A: Oblong leaf with attenuate base (normal), B: Oval leaf with obtuse base (0.06%), C: Larger size of flowers (0.1%), D: Compact inflorescence with dense flowering (0.04%), E: Abnormal flowering pattern (0.08%), F: Compact plant with short inflorescence and oval leaves (0.1%).

The leaf shape of treated plants showed an oval shape with obtuse base (Figure 2B) compared to the normal leaf shape of oblong and attenuated base (Figure 2A). Some mutants produced flowers in size (1.0 cm) twice as big as those of the control (0.5 cm) (Figure 2C). In the case of inflorescence, higher number of flowers and shortening of the flower stalks (5 - 7cm) were observed in treated plants compared to the control (45 cm) (Table 3, Figure 2D). The concentration of 0.08% also gave abnormal flower growth; *i.e*., top of inflorescence produced vegetative parts, and it continued to grow even after flowering time was over (Figure 2E). The compact and erect type of the inflorescence occurred often in treated plants while the control had long and drooping type of inflorescence (Table 3, Figure 2F).

Table 3 shows the characteristics of mutants and normal (control) plants. The results showed that mutagenic treatments produced much variability in the plants with some producing useful characteristics that were the objectives of this research. Plants with compact structure, oval leaves, larger flowers in higher numbers and short and compact inflorescences were obtained through this research.

Mutant generations

Both M1 V1 (first generation of mutants) and M_1 V_2 (second generation of mutants) plants produced flowers one month earlier than the control plants. The inflorescence lengths of M_1 V_2 plants were also shorter than those of the control. Most of the inflorescences were compact and of erect type and pattern of flowering was also not of truly raceme type. Compared to control plants which showed raceme flowering behavior, treated plants exhibited much variation in flowering. Their flower-opening pattern did not start from the base of the inflorescence, but sometimes from the middle and migrating in both directions. In some, it did not show a definite pattern.

CONCLUSIONS

The plants treated with colchicine showed changes in plant architecture. The leaves of treated plants showed an oval shape with shorter, rounded based leaves compared to the normal oblong shape. They also showed early flowering compared to the control, with shorter flower stalks (5 – 7 cm) compared to the control (45 cm). The compact and erect type of inflorescence occurred often in treated plants while the control had long and drooping type of inflorescence. All the dosages of gamma treatments resulted in poor growth patterns compared to the control plants, but the treated plants showed dark green colored leaves compared to the control. The treatment of 7 Krad gave flowers with different coloration of flower stalk and different flowering pattern. The results of this experiment showed that mutagenic treatments produced much variability in the plants with some producing useful characteristics that were the objectives of this research. Plants with compact structure, rounded leaves, larger flowers in higher numbers and better inflorescence characteristics were obtained through this research.

ACKNOWLEDGEMENTS

The authors thank Ms. Ranjani Peiris (HORDI, Ganoruwa) for providing irradiation facilities, and Ms. K.A.C.N. Senevirathne (Agricultural Monitoring Officer), Mr. A. Yogarajah (Research Assistant) and Mr. K.N. Yapa (Curator), Royal Botanic Gardens, Peradeniya, for their assistance and cooperation. The first author also would like to express her gratitude to TWOWS association for financial support during the course of this research and the Ministry of Agriculture and Irrigation, Myanmar for giving this valuable opportunity.

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