International Journal of Agriculture Innovations and Research
Volume 1, Issue 6, ISSN (Online) 2319-1473

Colchicine Content in Induced Mutants of Glory Lily (*Gloriosa superba* L.)

S. Anandhi
Ph.D. Scholar,
Deptt. of Medicinal and Aromatic Crops,
Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003,
Tamil Nadu, India
Email: sanandhijasmin@gmail.com
Phone : 0422-6611365

K. Rajamani
Professor and Head,
Deptt. of Medicinal and Aromatic Crops,
Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003,
Tamil Nadu, India
Email: rjmanicbe@rediffmail.com

M. Jawaharlal
Professor and Head,
Deptt. of Floriculture and Landscaping,
Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003,
Tamil Nadu, India
Email: jawaharflori@yahoo.com

M. Maheshwaran
Professor, Deptt. of Plant Breeding and Genetics,
Tamil Nadu Agricultural University,
Coimbatore-641 003, Tamil Nadu, India
Email: mahes@tnau.ac.in

R. Gnanam
Professor, Deptt. of Plant Molecular Biology and Biotechnology,
Tamil Nadu Agricultural University,
Coimbatore-641 003, Tamil Nadu, India
Email: rgnanam2000@yahoo.com

***Abstract* – An investigation was carried out to induce mutants in *Gloriosa superba* L. possessing high value alkaloid *viz*., colchicine and colchicoside, used in the treatment of gout, rheumatism and cancer. VM_2 generation was raised from EMS, DES and gamma ray treated VM_1 tubers of Glory lily. The mutants had apparent morphological changes in plant height, number of leaves and flowers as well yield characters. Five high yielding mutants *viz*., T_8P_2, $T_{10}P_1$, $T_{10}P_4$, T_7P_3, T_9P_4 were subjected to colchicine estimation using High Performance Liquid Chromotagrapy. These promising mutants seemed to possess high colchicine content, than the control. The maximum colchicine content of 0.707 per cent was observed in 2.00 per cent EMS (T_7P_3), followed by 0.702 per cent in 1.00 per cent DES (T_8P_2). The obtained results implicate that physical and chemical mutagens contribute to variations in alkaloid content.**

***Keywords* - Glory Lily, Colchicine, Mutation, Breeding, HPLC.**

I. INTRODUCTION

Gloriosa superba L., belongs to Colchicaceae family and popularly known as 'Glory Lily', 'Kalihari', 'Ognisikha' etc. Due to its wavy edged yellow and red flowers. It is a perennial climbing glabrous herb with tuberous rootstock. It is distributed throughout India and commercially cultivated in Tamil Nadu [1]. Seeds and tubers contain valuable alkaloids *viz*., colchicine and colchicoside as the major constituents, which cures gout and rheumatism. Due to the action of colchicine on spindle fibre formation during cell division, the plant has been identified as a potential anti-cancerous drug. In the Indian Systems of Medicine, the tubers are used as tonic, antiperiodic, antihelmenthic and also against snake bite [2]. Seeds are used for relieving rheumatic pain and as a muscle relaxant. The species is also widely believed to have medicinal properties [3].

The genetic variability of this species is low owing to the continued vegetative propagation through tubers which has reduced the vigour, tolerance to biotic and abiotic stress causing low yields. The growing demand for the seeds of *G. superba* in the international market and the wider popularity it has gained among the farmers necessitates attempts to induce new variability with high yield, high colchicine content, dwarf stature and leaf blight resistant of the plant as well.

Mutation breeding is envisaged as a complementary to conventional breeding due to the existence of polyploidy, crossing barriers, poor seed set and germination. Hence induced mutagenesis has become one of the accepted tools to improve the economic traits of the crop plants especially in vegetatively propagated crops like *G. superba*. As a result of following mutagenic treatments, a mixed bag of unexpected miracles of induced variations has been achieved in an array of horticultural crops [4]. Achievement during recent years in this field has highlighted the utility and usefulness of mutation breeding in crop improvement [5]. As the mutation study was aimed at producing high yielding mutants with high colchicine content, the present study was focused on the estimation of colchicine content in elite mutants of second generation vegetative mutants (VM_2) using High performance liquid chromatography.

II. MATERIALS AND METHODS

The *Gloriosa superba* genotype 'Mulanur", was selected as the original plant materials for mutational breeding experiments. Sprouted tubers of uniform size weighing 50-60 g were selected for mutagenic treatment. Tubers were subjected to three doses of gamma irradiation (0.50, 1.00, 1.50 kR at Gamma chamber - 900 installed at the Sugarcane Breeding Institute, Coimbatore), ethyl methyl sulphonate (1.0, 1.5 and 2.0 %) and diethyl sulphonate (1.0, 1.5 and 2.0 %). The shoots produced from the planted tubers following mutagenic treatment was represented as first vegetative generation; which was designated as VM_1 generation plants. Tubers collected from the VM_1 plants were planted in the field for second generation. Observations on the variation in biometrical and yield parameters were recorded on 120 and 180 days after planting (DAP) respectively in both the generations. Five

high yielding mutants were selected from VM_2 generation and the colchicine content was estimated using HPLC.

A. Estimation of colchicines-Sample preparation

Five hundred milligrams of dried and powdered seed samples were macerated with 25 ml of methanol at room temperature for 24 hours and sonicated for 45 minutes in an ultrasonic bath. The extract was filtered and adjusted to a final volume of 25 ml with methanol. An aliquot of the extract was filtered through 0.22 µm filter (Nylon Acrodisc 4427) before HPLC analysis [6].

B. Standards preparation

Pure colchicine supplied by SIGMA, supplied by Alchem International Ltd, New Delhi was used as reference substance. A stock solution containing 100 mg of colchicine standard in 10 ml HPLC-grade methanol was prepared separately for calibration and for the control standards.

C. HPLC Analysis

Quantitative determination of the alkaloids was carried out by RP-HPLC using a C18 column and a C18 precolumn packed with Kromasil. The mobile phase consisted of water and acetonitrile (60:40). A Hewlett-Packard series 1050 liquid chromatograph equipped with a quaternary pump system, a diode array detector operating at 350 nm, and data processing were used for the analyses. Amount of colchicine present in dry weight of sample was calculated using the following formula, given by [7] and expressed in per cent dry weight.

$$Cp(s) = \frac{Ap(s)}{Ap(st)} X\ Cp(st)$$

Cp (s) is the concentration of the solute in the mixture.

Ap (s) is the area of the peak for the sample in HPLC chromatogram.

Ap (st) is the area of the peak for the standard in HPLC chromatogram.

Cp (st) is the concentration of standard used for injecting in HPLC.

III. Results

A simple reversed-phase HPLC method with gradient elution was developed in order to determine colchicine in seeds. A typical HPLC chromatogram of the seed extract from *Gloriosa superba*. By measuring peak areas at 350 nm for colchicine, a calibration curve was constructed which was linear (r2 > 0.99) in the range 1.0–250 ppm. A control standards containing 70 ppm of colchicine was used to ensure accuracy and precision; their RSD (%) values were within 2 % of the actual concentrations. The limit of detection of the method was determined to be 50 ng/mL. The method described has the advantage of using a simple gradient elution in the reversed-phase mode without adding buffers. Addition of acetic acid to the mobile phase renders the method compatible with HPLC-MS requirements.

Data in Table 1 show that the physical and chemical mutagens significantly affected the colchicine content seeds of Gloriosa when compared with the untreated samples. The colchicine content (per cent dry weight) of seeds of five high yielding mutants were analysed using HPLC. The colchicine content was maximum (0.707 per cent) in T_7 P_3 of 2.00 per cent EMS and the minimum (0.537 per cent) was obtained from $T_{10}P_4$ of 1.50 per cent DES. The control (T_1P_1) recorded 0.168 per cent of colchicine (Fig.1, 2&3).

IV. Discussion

Induced mutations are one of the tools used to create wide variability. Since spontaneous mutations occurs at a very low frequency and often do not include the full range of variability, mutations are induced in high frequencies by physical or chemical mutagens [8]. Achievements in this field have highlighted the utility and usefulness of mutation breeding in crop improvement programme especially wherever there is a narrow genetic variability. Number of useful and potential viable mutants in many horticulture crops [9]. Wide ranges of physical and chemical mutagens are now commonly used for inducing mutations in many crop plants

Mutation breeding of vegetatively propagated plants have their unique advantages compared with other kind of crops and other breeding methods [10]. Firstly, the variation frequency increased greatly; which can be of hundred or thousand times higher than natural frequency. Secondly the target mutation appeared, the variation could be rapidly stabilized by vegetative propagation methods to speed up the selection process.

The medicinal utility of a particular plant species is related to the quantity of that marker compound. The medicinal importance of *G. superba* is due to the presence of alkaloids in all parts of the plant, mainly colchicine (superbine), an amino alkaloid derived from the amino acids phenylalanine and tyrosine.

In this investigation, the quality parameters *viz*., colchicine content was influenced by mutagenic treatment. The maximum colchicine content of 0.707 per cent was observed in 2.00 per cent EMS (T_7P_3), followed by 0.702 per cent in 1.00 per cent DES (T_8P_2). Higher doses of mutagen were directly proportional to the quality parameters of coleus. Enzymes involved in different stages of biosynthesis of colchicine might have triggered the production of precursors. Similarly the variations in the synthesis of colchicine might be due to promotive or inhibiting influence exerted by gamma rays and EMS at different dosages causing altered physiological and biochemical reactions in enzyme biosynthesis.

The mutagens would have enhanced protein synthesis and synthesis of cellulose hydrolyzing enzymes which might have triggered the secondary metabolite. Enzymes involved in different stages of biosynthesis of colchicine might have triggered the production of precursors. This is in accordance with the earlier works of [11] who found that diosgenin content increased at 2.00 kR gamma rays.

Enhanced forskohlin content (0.75 per cent) in tubers coleus was reported by [12] after 3.50 kR gamma rays + 1.50 % EMS treatment. Present findings were also in corroboration with findings of [13] in ginger, [14] in turmeric and [15] in sweet pepper. Vegetative slips of

Jamrosa were exposed to gamma rays by [16] results indicated that there was no increase in variability in growth parameters and oil content. But the oil samples in treated materials showed increased variation in composition of monoterpene, hydrocarbons, limonene and myrcene contents.

The colchicine being secondary metabolites besides the genetic factors may also be influenced by the environmental and seasonal factors. The relatively high colchicine content of the five elite mutants examined in this study has to be forwarded to the further generation for evaluating the stability. From the results, it is evident that there is wide variation in the biochemical compounds of Gloriosa after physical and chemical mutation, which can be further exploited to popularize the useful mutants for extraction of drugs.

REFERENCES

[1] Saxena OP, Brahmam M (1995). The Flora of Orissa. Pub: Regional Research Laboratory (now IMMT under CSIR), Bhubaneswar and Orissa Forest Development Corporation Ltd, Bhubaneswar, 3, 965-966.

[2] Gupta LM, Rana RC, Raina R, Meenakshi Gupta (2005). Colchicine content in *Gloriosa superba* L. Journal of Research, 4, 238-241.

[3] Farooqi AA, Kumaraswamy BK, Bojappa KN, Pusalkar VR, Gupta R (1993). Plantations of the clinically important *Gloriosa superba*. Indian Horticulture, 37, 26-29.

[4] Chopra VL (2005). Mutagenesis: Investigating the process and processing the outcome for crop improvement. Current Science, 89(2): 353-359.

[5] Mishra SN, Dixit S, Choudhary D (2006). Response of plants to heavy ions irradiation. Indian Journal Plant Physiology, 11(4): 333-348.

[6] Daniel G Omez, Jose Azorin, Jaume Bastida, Francesc Viladomat, Carles Codina (2003). Seasonal and spatial variations of alkaloids in *Merendera montana* in relation to chemical defense and phenology. J. Che. Eco., 29, 1117-1126.

[7] Scott RPW (1996). Chromatographic detectors: Design, function and operation. Chromatographic Science Series, 70. Marcel Dekker Inc. New York, Basel, Hong- Kong.

[8] Konzak CF, Nilan RA, Legault RR, Heiner RE (1961). Modification of induced genetic damage in seeds. Proc. Symp. Karisrune. I.A.E.A. and F.A.O. Vienna, pp. 150-169.

[9] Gustaffson A (1963). Productive mutation induced in barley by ionizing radiations and chemical mutagens. Heriditas, 50: 211-263.

[10] Zhao Shirong WJLL (2002). Induced Mutations for Improvement of Fruit Trees and Ornamental Plants in China. http://www.fnca.jp/english/e_old/2_totuzenheni/ 3/2002ws/04/01 china/main.html.

[11] Gupta MN, Lakshmi V, Dixit BS, Srivastava SN (1985). Morphological and biochemical investigations of *Dioscorea floribunda* after gamma irradiation. Int. J. Crude. Drug Research, 22 (2): 73-78.

[12] Velmurugan M (2007). Induced mutagenesis for improvement of yield and forskohlin content in coleus (*Coleus forskohlii* Briq.). Ph.D., Thesis, Tamil Nadu Agricultural University, Coimbatore.

[13] Jayachandran, Sethumadhava (1988). Effect of CCC, ethrel and kinetin as quality of Ginger. Agricultural Research Journal of Kerala, 26(2): 277-279.

[14] Ushanandhinidevi H (2004). Induction of mutagenesis in turmeric (*Curcuma longa* L.) through gamma rays for variability and quality improvement. Ph.D., Thesis, Tamil Nadu Agricultural University, Coimbatore

[15] Honda I, Kikuchi K, Matsuo S, Fukuda M, Sai H, Ryuto H, Fukunishi N, Abe T (2006). Heavy ion induced mutants in sweet pepper isolated by M_1 plant selection. Euphytica, 152(1): 61-66.

[16] Singh C, Pal S, Lal S, Thappa RK, Kaul BL (1991). Gamma ray induced alternations of growth and oil composition in Jamrosa. In: Intl. Sym. on newer trends in Essential oil and Flavours, RRL, Jammu and Kashmir, pp. 56.

Table 1: Effect of mutagens on colchicine content (% dry weight) in seeds in VM2 generation of glory lily

S. No.	Mutants	Treatment	Colchicine content (% dry weight)
1.	T_1P_1	Control	0.168
2.	T_8P_2	1.00 % DES	0.702
3.	$T_{10}P_1$	1.50 % DES	0.561
4.	$T_{10}P_4$	1.50 % DES	0.537
5.	T_7P_3	2.00 % EMS	0.707
6.	T_9P_4	1.25 % DES	0.573
Mean			0.541
SE			0.073

T-Treatment; P-Plant number

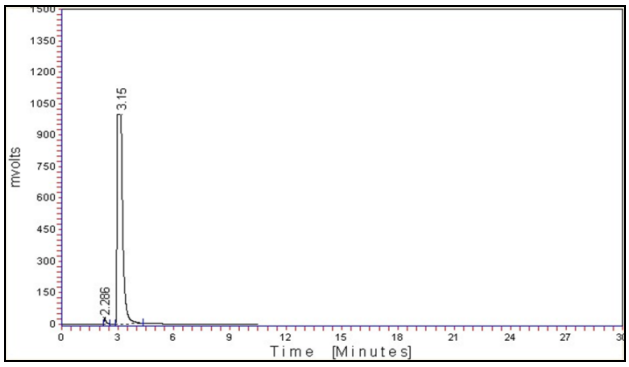

Fig.1. HPLC Chromatogram of Colchicine standard

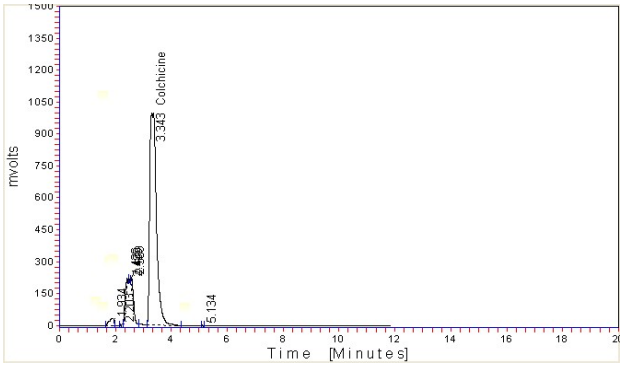

Fig.2. HPLC chromatogram of high colchicine content in seeds of the mutant T7P3

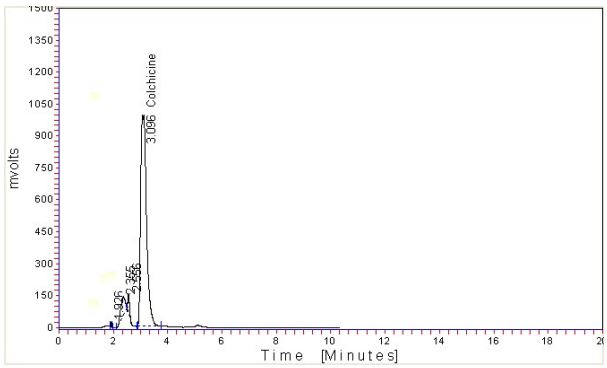

Fig.3. HPLC chromatogram of high colchicine content in seeds of the mutant T8P2