International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(4): 1114-1117
© 2017 IJCS
Received: 08-05-2017
Accepted: 09-06-2017

M Lakshmi Madhuri
College of Horticulture, Dr. Y S R Horticultural University, Venkataramannagudem, Andhra Pradesh, India

MLN Reddy
College of Horticulture, Dr. Y S R Horticultural University, Venkataramannagudem, Andhra Pradesh, India

AVD Dorajee Rao
College of Horticulture, Dr. Y S R Horticultural University, Venkataramannagudem, Andhra Pradesh, India

K Giridhar
College of Horticulture, Dr. Y S R Horticultural University, Venkataramannagudem, Andhra Pradesh, India

K Uma Krishna
College of Horticulture, Dr. Y S R Horticultural University, Venkataramannagudem, Andhra Pradesh, India

Correspondence
M Lakshmi Madhuri
College of Horticulture, Dr. Y S R Horticultural University, Venkataramannagudem, Andhra Pradesh, India

Induction of variability for leaf characters through physical and chemical mutagens in turmeric cv. prathibha

M Lakshmi Madhuri, MLN Reddy, AVD Dorajee Rao, K Giridhar and K Uma Krishna

Abstract
The present investigation was conducted during *kharif* 2016-2017 at College of Horticulture, Dr. Y.S.R. Horticultural University, Venkataramannagudem to assess the impact of mutagens on leaf characters like leaf length, leaf width, leaf chlorophyll and leaf curcumin in turmeric. The experiment was laid out in factorial randomized block design and replicated thrice under open field condition. Rhizomes are treated with gamma rays (25 Gy and 50 Gy) and EMS (250 ppm, 500 ppm and 1000 ppm) along with control. The plants recorded maximum leaf length, width, chlorophyll content and curcumin with 25 Gy, 250 ppm EMS and combination of 25 Gy + 250 ppm EMS. Among the treatments, 25 Gy gamma rays and 250 ppm EMS concentration resulted in creating variability in turmeric.

Keywords: turmeric, gamma rays, ems and leaf characters

Introduction
Turmeric (*Curcuma longa* L.) is one of the important spices grown in India, which plays an important role in the national economy. It is a member of the family, Zingiberaceae and originated from South East Asia. States like Telangana, Andhra Pradesh, Orissa, Kerala, Tamil Nadu, Karnataka, Maharashtra and West Bengal are in the forefront in turmeric cultivation and research. Cultivated turmeric, *Curcuma longa* L. is considered to be a triploid with a somatic chromosome number of sixty three (2n= 3x=63) (dibasic amphidipliod) and sets seeds rarely. Since, turmeric is an asexually propagated crop with no regular seed production, clonal selection is the major mode of crop improvement. The first step in the crop improvement of this clonally propagated crop is to exploit the variability existing among the land races, create more variability through mutation and somaclonal variation. The use of mutagens for inducing variability assumes greater significance. In vegetatively propagated crops with reproductive sterility (such as turmeric), mutation breeding this is very important tool in crop improvement (Broertjes and Van Harten, 1988) [1]. An attempt was therefore made to induce variability for leaf characters like leaf length, leaf width, leaf chlorophyll content and leaf curcumin content by gamma rays and EMS.

Material and Methods
The present investigation was carried out during *Kharif*, 2016-2017 at College of Horticulture, Dr. Y.S.R. Horticultural University, Venkataramannagudem, West Godavari District, Andhra Pradesh. The experiment was laid out in factorial randomized block design with 12 treatments and replicated thrice under open field condition. Healthy and disease free fingers with well-developed buds were selected for planting. Prathibha variety is used as a planting material. Rhizomes are treated with gamma rays (25 Gy and 50 Gy) and EMS (250 ppm, 500 ppm and 1000 ppm) along with control. Treated rhizomes are planted in protrays. After one month, seedlings are transplanted to main field. After planting basal dose of NPK fertilizers were applied at the rate of 150:125:250 Kg/ha. The nitrogenous and potash fertilizers were applied at three equal doses at 45, 90 and 135 days after planting in the form of Urea, MOP and Phosphate fertilizer was applied in the form of SSP. The field was irrigated before planting. Light irrigation was given on the third day after transplanting. Therefore, irrigation was given at weekly intervals depending on weather and soil conditions. Five plants in each treatment were tagged randomly for recording observations and mean values were subjected to statistical scrutiny.

Leaf Characters

Leaf length (cm)

Length of fully opened leaf was measured with a measuring scale at 60, 90, 120, 150 and 180 days after planting and expressed in centimeters.

Leaf width (cm)

Width of the leaf at the widest part of the leaf was measured with a scale and expressed in centimeter.

Leaf chlorophyll content (SPAD Readings)

Leaf chlorophyll content was measured by using SPAD meter.

Leaf curcumin content (%) at 150 DAP of 1st fully opened leaf from top

The curcumin content was estimated by adopting the method given by Manjunath *et al.* (1991) [5]. The dried leaves were grounded to the fine powder and 0.1 g of grind leaf powder was taken and mixed with 40 ml of distilled alcohol and kept it for 2 hours 30 minutes. Then the extract was transferred to a 100 ml volumetric flask and volume made with alcohol. Later, it was filtered and then an aliquot of 5 ml was transferred into a 100 ml volumetric flask, made the volume with alcohol and mixed the aliquot thoroughly. The absorbance of solution was measured at 425 nm against alcohol blank. Using absorbance value of standard solution, curcumin content was calculated by adopting the following formula

$$\text{Curcumin content} = \frac{0.0025 \times A\ 425 \times \text{Volume made up} \times \text{Dilution factor} \times 100}{(G/100g)\ \text{Absorbance of standard (0.42)} \times \text{weight of samples} \times 1000}$$

Since, 0.42 absorbance at 425 nm corresponds to 0.0025 g curcumin

Results and Discussion

Leaf length (cm)

The length of leaf varied significantly with doses of gamma rays, EMS concentrations and their interactions.

Among the different doses of gamma rays, 25 Gy recorded higher leaf length (31.19 cm). In EMS concentrations, maximum leaf length (31.70 cm) was recorded with 250 ppm EMS concentration and minimum leaf length (28.90 cm) was recorded with1000 ppm EMS concentration at 150 DAP. Among treatment combinations, 25 Gy gamma rays + 250 ppm EMS concentration showed highest leaf length (32.03 cm) at 150 DAP. Lowest leaf length (28.43 cm) was recorded in 50 Gy gamma rays + 1000 ppm EMS concentration. However, control (0 Gy gamma rays + 0 ppm EMS) was recorded highest leaf length (38.13 cm) than any of the above mutagenic treatments (Table 1).

Leaf width (cm)

Application of mutagens showed significant variation in the leaf width with different gamma rays doses, EMS concentrations and their interactions at all stages of plant growth. However, the mean leaf width increased from 8.68 cm at 60 DAP to 11.97 cm at 150 DAP (Table 4.8).

In gamma ray doses, maximum leaf width (11.87 cm) was recorded in 25 Gy gamma rays. In EMS, increased leaf width (12.01 cm) was noticed in 250 ppm EMS concentration and decreased leaf width (11.08 cm) was observed with 1000 ppm EMS concentration. Among treatment combinations, 25 Gy gamma rays + 250 ppm EMS concentration recorded maximum leaf width (12.16 cm) at 150 DAP. Minimum leaf width (10.76 cm) was recorded in 50 Gy gamma rays + 1000 ppm EMS concentration. Whereas control, no mutagenic effect recorded higher leaf width (15.46 cm) at 150 DAP (Table 2).

Leaf chlorophyll at 120 DAP (SPAD)

There was a reduction in chlorophyll content with increase in the doses of physical or chemical mutagenic treatments. Among the different doses of gamma rays, 25 Gy gamma rays recorded maximum chlorophyll content (22.15) at 120 days after planting. Among the EMS concentrations, the highest chlorophyll content (22.65) was recorded with 250 ppm EMS concentration and lowest chlorophyll content was recorded with 1000 ppm EMS concentration (20.00) at 120 days after planting. Among the treatment combinations, 25 Gy gamma rays + 250 ppm EMS concentration showed highest chlorophyll content (22.69) and lowest chlorophyll content (19.96) was recorded in 50 Gy gamma rays +1000 ppm EMS concentration. However, control (0 Gy gamma + 0 ppm EMS) was recorded highest leaf chlorophyll (26.06) than any of the above mutagenic treatments (Table 3).

Leaf curcumin content (%) at 150 DAP

Leaf curcumin content decreased with an increase in the doses of gamma rays and EMS concentrations. Gamma rays treatment, 25 Gy recorded higher leaf curcumin (2.05%). Among the EMS concentrations, highest leaf curcumin (2.10%) was recorded with 250 ppm EMS concentration and lowest leaf curcumin of 1.83 per cent was noted in 1000 ppm EMS concentration. Among the treatment combinations, 25 Gy gamma rays + 250 ppm EMS concentration recorded maximum leaf curcumin (2.10%) and minimum leaf curcumin (1.70%) was recorded at 50 Gy gamma rays + 1000 ppm EMS concentration. However, control (0 Gy gamma + 0 ppm EMS) was recorded highest leaf curcumin (2.50%) than any of the above mutagenic treatments (Table 4).

In the present investigation, gamma rays 25 Gy and EMS 250 ppm, combination 25 Gy + 250 ppm recorded the maximum leaf length and leaf width. Reduced height of plant and tiller production might be the contributory factors for reduction in the leaf characters. It may also be due to the direct effect of the gamma rays on the growing points. In the cells of growing shoot, mitotic and meiotic aberrations occur during mutation which may cause inhibitory effect on growth rate. Inhibition of vegetative growth may be due to radiation effect on the chromosomal material, genetic injury induced in dividing cells and deficiency of some physiological pre requisites to the process of cell division was reported by Natarajan (1975) [6] in turmeric.

Gamma rays 25 Gy and EMS 250 ppm, combination 25 Gy + 250 ppm recorded the maximum chlorophyll content. The reduction in chlorophyll content with increase in the doses of mutagenic treatments either through physical or chemical means might be due to the inhibitory effect of them which was in turn due to various mitotic and meiotic aberrations as mentioned earlier. The reduced production of chlorophyll molecules also might be perhaps due to radiation effect on the

chromosomal material, genetic injury induced in dividing cells and deficiency of some physiological pre requisites to the process of cell division as was reported by Natarajan (1958) [7] in turmeric, Gupta *et al*. (1982) [4] in *Costus* and Giridharan (1984) [2] in ginger, Giridaran and Bala krishnan (1992) [3].

Gamma rays 25 Gy and EMS 250 ppm, combination 25 Gy + 250 ppm recorded the maximum leaf curcumin content. Leaf curcumin content decreased with an increase in the doses of gamma rays and EMS concentrations. The biosynthesis of curcumin involves various steps hastened by different enzymes. The phenyl propanoid pathway is the prime route for the synthesis of this xanthophyll pigment, curcumin. Enzymes involved at different stages for biosynthesis of curcumin would have become more active thereby resulting in higher curcumin content at lower doses of gamma rays. Anatomical features namely central cylinder and cortex with more congregations of specialized cells which are the oleoresin bearing cells act as the site of curcumin accumulation. These specialized cells might have been decreased at higher doses of gamma rays thereby affecting accumulation of curcumin at higher doses. This is in accordance with the earlier works of Gupta *et al*. (1982) [4] who found that diosgenin content increased at 2.0 kr, whereas it decreased at 3.0 kr in *Costus*.

Table 1: Leaf length (cm) as influenced by mutagens in turmeric cv. Prathibha

Gamma Irradiation (Gy)(A) / EMS Concentration (ppm) (B)	60 DAP				90 DAP				120 DAP				150 DAP			
	0	25	50	Mean	0	25	50	Mean	0	25	50	Mean	0	25	50	Mean
0	26.06	24.00	22.03	24.03	30.00	27.00	25.03	27.34	34.13	29.90	28.16	30.73	38.13	32.90	31.03	34.02
250	23.40	22.69	21.86	22.65	26.60	26.00	24.93	25.84	29.63	29.00	27.76	28.80	32.63	32.03	30.43	31.70
500	21.63	21.91	21.46	21.67	24.83	24.86	24.50	24.73	27.93	27.90	27.46	27.76	30.90	30.86	30.33	30.70
1000	20.03	20.00	19.96	20.00	23.00	22.93	22.73	22.88	26.20	26.03	25.50	25.91	29.30	28.96	28.43	28.90
Mean	22.78	22.15	21.33	22.08	26.10	25.20	24.30	25.2	29.47	28.20	27.22	28.29	32.74	31.19	30.05	31.32
	S Em±		CD at 5%		S Em±		CD at 5%		S Em±		CD at 5%		S Em±		CD at 5%	
A	0.28		0.84		0.28		0.84		0.28		0.84		0.29		0.85	
B	0.33		0.97		0.33		0.98		0.33		0.97		0.33		0.98	
A × B	0.57		168		0.57		1.69		0.57		1.68		0.58		1.71	

Table 2: Leaf width (cm) as influenced by mutagens in turmeric cv. Prathibha

Gamma Irradiation (Gy) (A) / EMS Concentration (ppm) (B)	60 DAP				90 DAP				120 DAP				150 DAP			
	0	25	50	Mean	0	25	50	Mean	0	25	50	Mean	0	25	50	Mean
0	9.63	9.43	9.00	9.35	11.43	10.70	9.96	10.70	13.43	11.73	11.00	12.05	15.46	12.70	12.03	13.40
250	9.16	9.00	8.50	8.88	10.36	10.10	9.36	9.94	11.40	11.13	10.40	10.97	12.43	12.16	11.43	12.01
500	8.66	8.53	8.10	8.43	9.60	9.43	9.03	9.35	10.63	10.46	10.06	10.38	11.66	11.50	11.10	11.42
1000	8.26	8.03	7.90	8.06	9.30	9.06	8.70	9.02	10.33	10.10	9.73	10.05	11.36	11.13	10.76	11.08
Mean	8.93	8.75	8.37	8.68	10.17	9.82	9.26	9.75	11.45	10.85	10.30	6.52	12.73	11.87	11.33	11.97
	S Em±		CD at 5%		S Em±		CD at 5%		S Em±		CD at 5%		S Em±		CD at 5%	
A	0.04		0.12		0.06		0.19		0.06		0.20		0.06		0.20	
B	0.05		0.14		0.07		0.22		0.07		0.23		0.08		0.23	
A × B	0.08		N.S.		0.13		0.38		0.13		0.40		0.13		0.40	

Table 3: Leaf chlorophyll as influenced by mutagens in turmeric cv. Prathibha

Gamma Irradiation (Gy) (A) / EMS Concentration (ppm) (B)	Leaf chlorophyll (SPAD)			
	0	25	50	Mean
0	26.06	24.00	22.03	24.03
250	23.40	22.69	21.86	22.65
500	21.63	21.91	21.46	21.67
1000	20.03	20.00	19.96	20.00
Mean	22.78	22.15	21.33	22.08
	S Em±		CD at 5%	
A	0.28		0.84	
B	0.33		0.97	
A × B	0.57		1.68	

Table 4: Leaf curcumin content (%) as influenced by mutagens in turmeric cv. Prathibha

Gamma Irradiation (Gy)(A) / EMS Concentration (ppm) (B)	Leaf curcumin (%)			
	0	**25**	**50**	**Mean**
0	2.50 (9.09)	2.30 (8.71)	2.20 (8.52)	2.33 (8.77)
250	2.20 (8.52)	2.10 (8.32)	2.00 (8.12)	2.10 (8.32)
500	2.10 (8.32)	2.00 (8.12)	1.90 (7.92)	2.00 (8.12)
100	2.00 (8.12)	1.80 (7.70)	1.70 (7.48)	1.83 (7.77)
Mean	2.20 (8.51)	2.05 (8.22)	1.95 (8.01)	2.06 (8.24)
	S Em±		CD at 5%	
A	0.03		0.09	
B	0.03		0.10	
A × B	-		N.S.	

Figures in the parenthesis indicate arc sine transformed values

References

1. Broertjes C, Van Harten AM. Application of mutation Breeding methods in the Improvement of vegetatively Propagated crops. An interpretive literature review, 1988.
2. Giridharan MP. Effect of gamma irradiation in ginger (Zingiber officinale. Rosc.) M.Sc. (Hort.) Thesis submitted to the Kerala agricultural university, Vellanikkara, India, 1984.
3. Giridharan MP, Balakrishnan S. Gamma ray induced variability in vegetative and floral characters in ginger. Indian Areca nut, Spices and Cocoa Journal. 1992; 15:68-72.
4. Gupta MN, Lakshmi V, Dixit VS, Srivastava SN. Gamma ray induced variability in Costus speciosus. Progressive Horticulture. 1982; 14:193-97.
5. Manjunath MM, Sattigeri VV, Nagaraj KV. Curcumin in turmeric. Spices India. 1991; 4(3):7-9.
6. Natarajan ST. Studies on the yield components and gamma ray induced variability in turmeric (Curcuma longa L.). M.Sc. (Ag.) Thesis submitted to faculty of horticulture, TNAU, Coimbatore, 1975.
7. Natarajan AT. A cytogenetically study of the effects of mutagens on plants with special reference to the induction of mutation. Ph.D. Thesis submitted to the Delhi University, India, 1958.