J. Hortl. Sci.
Vol. 4 (1): 41-44, 2009

Induction of mutation in Rough lemon (*Citrus jambhiri* Lush.) using gamma rays

H.K. Saini and M.I.S. Gill
Department of Horticulture
Punjab Agricultural University
Ludhiana – 141 004, India
E-mail: tegisaini@yahoo.com

ABSTRACT

The present investigation was carried out to study variability induced by gamma rays with respect to vegetative characters and LD_{50} dose in Rough lemon. Rough lemon seeds were gamma irradiated at doses of 0, 4, 6 and 8 kr along with control. Seed germination decreased with increasing dose of gamma radiation. Seedling height and leaf size also decreased with increasing dose of gamma radiation, whereas, apical branching, number of branches/seedling, number of variegated / albino seedlings and number of leaves increased with increasing dose of gamma radiation. Maximum variability for seedling height, number of leaves, leaf size, colour, internode length, and per cent apical branching was observed at two months from sowing in seeds treated with 8 kr dose of gamma radiation. Variability for all characters was, however, found to be minimum in the control.

Keywords: Gamma rays, citrus, rough lemon, variability, seeds

INTRODUCTION

In vitro mutagenesis is a valuable tool for improvement of a crop, especially when these is a need to add one or two easily identifiable characters in an otherwise well - adapted variety, without disturbing its basic genotype. At current levels of plant breeding research, mutation breeding is highly suitable, when natural variation does not provide gene(s) for desired traits. Mutation breeding is more effective than hybridization even when desired genes are present, but, tightly linked to undesirable genes. Frequency of spontaneous mutation is quite low (approximately 10-6 for an individual gene), hence, attempts have been made to accelerate the rate artificially using physical and chemical mutagens.

Attempts to induce variability in citrus have been made by various workers with desirable results like seedlessness in sweet orange and grapefruit cultivars (Davis and Albrigo, 1994) and salt tolerance in Troyer citrange (Garcia-Augustin and Primo-Millo, 1995). However, in rough lemon no specific information is available about LD_{50} dose and the degree and direction of variation caused. In the present study, variability induced by gamma rays was studied with respect to vegetative characters and LD_{50} dose in Rough lemon seeds.

MATERIAL AND METHODS

The present research was conducted at the Tissue Culture Laboratory, Department of Horticulture, PAU, Ludhiana, during 2007-08. Seeds from healthy fruits of Rough lemon were collected in August-September and exposed to gamma rays (after air-drying) at dosage of 0, 4, 6 and 8 kr from ^{60}Co source emitting 110 kr per hour. A hundred seeds were cultured on MS medium and each treatment was replicated thrice, so that there were three hundred seeds receiving each treatment. Emerging seedlings were counted, at 10 day interval from sowing. LD_{50} dose was determined from the number of seed germinated upto 45 days from sowing, as, no seedling emerged after this period.

In vitro grown two-month old seedlings were used for measuring various growth parameters like height, internode length, number of leaves per seedling, and, leaf length and width, number of apical shoots per seedling, per cent apical shooting and per cent variegated and albino seedlings were recorded. Data were analyzed as per completely randomized block design (Snedecor and Cochran, 1999).

RESULTS AND DISCUSSION

Germination of seeds was severely affected with increasing dose of gamma radiation. At 45 days from sowing, seed germination was maximum in control (63.45%), followed by 4 kr (58.48%) treatment. LD_{50} value was observed at 8 kr. Seed germination decreased with increase in dose of gamma-radiation. Similar results were reported by Gregory and Gregory (1965) using X-ray treatment and Hearn (1984) with gamma radiation in citrus.

Data on seedling height in gamma irradiated rough lemon seeds after two months of sowing are presented in Table 1. Maximum seedling height was observed in control (7.79 cm) and minimum in 8 kr (3.76 cm) treatment (Fig 1a). Seedling height in different gamma ray treatments ranged from 5.8-9.0 cm in the control, 5.3-8.8 cm in 4 kr, 2.0-9.4 cm in 6kr and 0.4-7.0 cm in 8 kr treatment. Likewise, Kerkadzi (1985) observed decrease in seedling height with increasing gamma radiation dose in citrus. Reduction in height was also reported by Legave *et al* (1989) and Waqar *et al* (1992) in kinnow seedlings.

A majority of the seedlings in control were of medium height, while, in 8kr treatment seedlings were dwarf. The proportion of dwarf seedlings varied from none in control to 64.90% in 8 kr treatment. In the medium height category, proportion of seedling varied from 35.37% in 8 kr to 100% in control, whereas, their proportion ranged from none (control, 4 kr and 8 kr) to 12.43% in 6 kr treatment under the tall seedlings category.

Radiation treatments probably induced some changes at the gene level that ultimately reflected in substances that trigger biochemical processes controlling different aspects of growth. These substances, identified as auxins, gibberellins, ethylene and abscisic acid called phytohormones, initiates biochemical reactions and induce changes in chemical patterns that lead to various modifications and variations in plant characters, viz., height, branching and stem thickness, as reported by Whittwer (1971).

Significant reduction in leaf size was found with increasing dose of gamma rays (Table 2) and, thus, the minimum leaf size (length 0.93 and breadth 0.43 cm) was observed in 8 kr treatment, followed by 6 kr.

Variability for leaf colour was maximum in 8 kr treated seedlings, while, it was minimum in control (Table 2, Fig 1c). The proportion of variegated leaves varied from none in control to a maximum of 59.54% in 8 kr treatment. In the albino category, the proportion varied from none

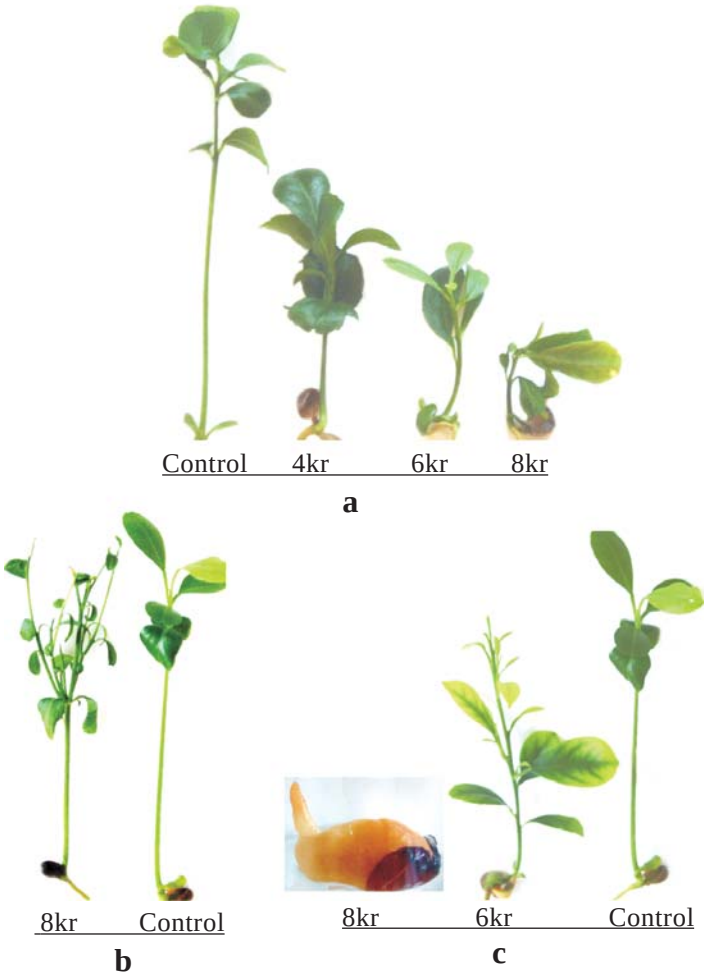

Fig 1. Effect of different doses of gamma radiation on (a) seedling height (b) branching and (c) leaf colour

Table 1. Effect of different doses of gamma rays on germination and height in Rough lemon seedling

Treatment	Germination (%)	Seedling height (cm)		Per cent seedlings in each category		
		Average	Range	Low<3 cm	Medium 3-9 cm	High >9 cm
Control	80.00 (63.45)*	7.79	5.8-9.0	0.00 (0.00)	100.00 (89.96)	0.00 (0.00)
4 kr	68.60 (58.48)	6.70	5.3-8.8	44.75 (41.96)	52.75 (46.56)	0.00 (0.00)
6 kr	65.80 (53.58)	5.52	2.0-9.4	33.53 (35.37)	55.12 (47.92)	12.43 (20.63)
8 kr	55.62 (50.06)	3.76	0.4-7.0	64.90 (53.65)	35.37 (36.48)	0.00 (0.00)
CD (*P=0.05*)	7.31	1.55	1.25	1.37	—	—

* Figures in parentheses are transformed values

Table 2. Effect of different doses of gamma rays on number of leaves, leaf size and leaf colour in Rough lemon seedling

Treatment	Number of leaves /seedling	Leaf size		Leaf colour (%)		
		Length (cm)	Breadth (cm)	Variegated	Albino	Normal
Control	2.23	2.07	1.07	0.00 (0.00)*	0.00 (0.00)	100.00 (89.96)
4 kr	9.73	1.80	0.85	15.16 (22.90)	0.00 (0.00)	79.69 (63.30)
6 kr	9.63	1.21	0.61	73.79 (59.54)	11.65 (19.94)	63.02 (52.52)
8 kr	11.30	0.93	0.43	31.09 (33.87)	0.00 (0.00)	47.14 (43.34)
CD (*P=0.05*)	1.86	0.43	0.13	7.03 __	3.60	

* Figures in parentheses are transformed values

(control, 4 kr and 6 kr) to 19.34% in 8 kr treatment. In the normal leaf category, proportion varied from 43.34% in 8kr treatment to 100 per cent in control.

The maximum average number of leaves per seedling 2 months from sowing was observed in 8 kr treatment (8.20), followed by 6 kr (7.60), whereas, the minimum average number of leaves per seedling was observed in the control (Table 2).

Swaminathan (1965) reported that besides causing various phytohormones to malfunction and cause changes in chemical patterns leading to morphological variations, radiation treatments also caused quantitative and qualitative alteration in hereditary material. Morphological effects due to radiation treatment have been reported in leaves and branches (Sparrow and Gunckel, 1956). These are generally recessive to the normal type or the condition they arise from, thereby suggesting that mutations induced are due to destruction of the gene.

Non-significant results were obtained for internode length in gamma ray treated Rough lemon seedlings. The present findings are in conformity with Jawaharlal *et al* (1991) in acid lime, thereby, indicating varietal or genetic specificity of each genotype to radiation. Most of the ill-effects of gamma radiation treatment started immediately after treatment and were manifest in terms of decreased sprouting capacity with increasing the dose.

Table 3. Effect of different doses of gamma rays on internode length, apical branching and number of branches/seedling in Rough lemon

Treatment	Internode length (cm)	Apical branching (%)	Number of branches/ seedling
Control	0.42	0.00(0.00)*	0.00
4 kr	0.56	44.40(41.76)	1.10
6 kr	0.63	11.00(19.34)	1.66
8 kr	0.93	5.00(12.87)	3.90
CD *(P= 0.05)*	NS	1.54	0.19

*Figures in parentheses are transformed values

With increase in the dose of gamma rays, there was increase in per cent apical branching and the number of apical branches per seedling (Table 3, Fig 1b). Maximum per cent branching was observed in 8 kr (41.76%) treatment with (3.90) apical branches per seedling, followed by 6 kr (19.34%) with 0.66 apical branches per seedling.

Results indicate that Gamma rays at doses of 6 and 8 Kr can be used to create sufficient variability in Rough lemon genotypes. These mutants can be further exploited for abiotic and biotic stress tolerance.

REFERENCES

Davis, F.S. and Albrigo, I.G. 1994. Citrus. CAB International, Wallingford, UK, pp 254

Garcia-Augustin, P. and Primo-Millo, E. 1995. Selection of a NaCl tolerant citrus plant. *Pl. Cell Rep*., **14**: 314-18

Gregory, W.C. and Gregory, M.P. 1965. Induced mutation in quantitative characters. Experimental basis for mutations to hardiness in citrus. *Proc. Soil Crop Sci. Soc. Fla*., **25**:372-76

Hearn, C.J. 1984. Development of seedless orange and grapefruit cultivars through seed irradiation. *J. Amer. Soc. Hortl. Sci*., **109**:270-73

Jawaharlal, M., Sambandamoorthy, S. and Irulappan, T. 1991. Effect of gamma-ray and EMS on seed germination and seedling growth in acid lime (*Citrus aurantifolia* Swingle). *South Ind. Hort*., **39**:332-36

Kerkadzi, I.G. 1985. Induced mutations in subtropical crops. V. Biological and genetic effect of treating citrus with gamma-radiation. *Subtrop Kul*., **4**:104-10

Legave, J.M., Tisne-Agostini, D. and Jacquemond, C. 1989. Physiological effects induced by acute gamma-irradiation of Clementine (*Citrus reticulata* Blanco). *Fruits Paris*., **44**:329-33

Snedecor CW and Cochran WG. 1999. Statistical methods. 6th edition. Oxford and IBH Publ. Co, Calcutta, pp 593

Sparrow, A.H. and Gunckel, J.E. 1956. The effect of plants on chronic exposure to gamma-radiation from radio cobalt. Proc Int Conf Peaceful Uses of Atomic Energy, **17**:52-59

Swaminathan, M.S. 1965. A comparison of mutation induction in diploid and polyploids. *Radiation Bot*., **5**:619-41

Waqar, A., Wasim, A. Farooqi and Sattar (Jr), A. 1992. Effect of gamma irradiation on the morphology of *Kinnow* seedlings. Proc First Intl. Seminar on Citriculture in Pakistan, 2-5 Dec, 1992

Whittwer, S.H. 1971. Radiation induced mutation in crop plants. *Outlook Agri*.,**6**:205-17

(MS Received 4 November 2008, Revised 18 February 2009)