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Effect of mutagens on *in vitro* seed germination and growth of rough lemon (*Citrus jambhiri*) seedlings

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ABSTRACT

An experiment was conducted during 2008–09 at Ludhiana to study the effect of various mutagenic treatments on germination and growth of rough lemon (*Citrus jambhiri* Lush.) seedlings. Developmental characteristics of the *in vitro* derived plantlets from treated seeds employing gamma radiation at 0, 20, 40, 60, 80, 100 and 120 Gy and the alkylating agent ethyl-methane sulfonate at 0, 0.2, 0.4, 0.6, 0.8 and 1.0% (v/v) were evaluated. Seeds were sown in Murashige and Skoog nutrient medium under controlled laboratory conditions (25±2°C, 16 hr photoperiod, 2000 lux).The dose required to kill half of the tested population (LD_{50})corresponded to 62 Gy for gamma radiation and 0.64% for ethyl methane sulphonatetreatment. Number of days taken for seedling emergence increased with increasing dose of gamma irradiation and ethyl methane sulphonate. Seed germination,seedling height,internodal length, number of leaves, leaf area, number of branches, root length and number of secondary roots decreased with increasing dose of gamma radiation and ethyl methane sulphonate.

Key words: Ethyl methane sulphonate, Gamma rays, Germination, *In vitro*, Rough lemon

Mutation breeding has been used in recent years as a valuable supplement to the method of plant breeding in the development of better crop cultivars (Arora and Pahuja 2008). For the improvement of a crop, the extent of genetic variability is more important than the total variability. The inheritance of important economic traits, such as yield, quality,adaptation, pest and stress resistance, upon which much of the future of plant improvement depends can be understood through the analysis of a wide range of induced mutations. Several workers have attempted for induction of mutation in citrus species using eitherphysical or chemical mutagens for evolving new citrus genotypes (Gulsen *et al.* 2007, Latado *et al.* 2006). In rough lemon (*Citrus jambhiri* Lush.) no specific information is available about LD_{50} dose and about the degree and direction of variation caused.In the present study,gamma rays and ethylmethane sulphonate have been used to study the effects of various mutagenic treatments on seed germination and different growth parameters of rough lemon seedlings.

MATERIALS AND METHODS

Seeds of rough lemon were obtained from citrus germplasm block of College Orchard of the University, Ludhiana, were employed in the mutagenesis treatments. The experiments were carried out in the Tissue Culture Laboratory during 2008–09. Five increasing dose of gamma irradiation (0, 40, 60, 80, 100 and 120 Gy) and ethyl methane sulphonate (0, 0.2, 0.4, 0.6, 0.8 and 1.0% v/v) were evaluated for seeds of rough lemon fully randomized in 5 replicates of 24 seeds (n = 120).

Dry seeds were submitted to gamma rays from the $Cobalt^{60}$ source. After removing the seed coat of treated seeds along with control, seeds were surface sterilized under Laminar Air Flow Cabinet with mercuric chloride (0.1%) solution for 4 min. and then the seeds were rinsed thrice with autoclaved distilled water to remove the traces of mercuric chloride. After sterilization, 3 seeds were sown in each culture jars containing solidified Murashige and Skoog (1962) nutrient medium under controlled laboratory conditions (25±2°C, 16 hr photoperiod, 2000 lux).

Chemical mutagenesis was carried out by immersing the seed in filter (0.22 micron Millipore) sterilized ethyl methane sulphonate at concentrations of 0 (control), 0.2, 0.4, 0.6, 0.8 and 1.0% for 4 hr in an incubator shaker (25+1°C, 70 rpm). The seed coats of the seed used in this experiment were removed and seeds were surface sterilized under Laminar Air Flow Cabinet with mercuric chloride (0.1%) solution for 4 min. and then 3 rinsing with autoclaved distilled water

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to remove the traces of mercuric chloride. Treated seeds along with control (120 seeds/treatment) were sown in culture jars containing solidified MS nutrient mediumunder controlled laboratory conditions (25±2p C, 16 hr photoperiod, 2000 lux). At the time of treatment the seeds had 44.23% moisture content obtained by drying the seeds at 60°C till constant weight.

In both sets of experiments, data were recorded for number of days taken to seed germination, per cent seed germination, seedling height, internodal length and number of leaves. Leaf area was measured by leaf area meter. LD_{50} doses were optimized by taking into account the germination of seedlings. The forecast analysis (Microsoft Excel) was used to calculate the lethal mutagen dosage required to kill half of the studied population (LD_{50}). Per cent germination was calculated by standard procedure 60 days after sowing. Seedling height and internodal length was measured with Vernier's calliper, 120 days after sowing. The experiment was laid in completely randomized design as per Singh *et al.* (1998). Microsoft Excel was used for statistical analysis.

RESULTS AND DISCUSSION

Seed germination attributes were significantly influenced by the dose of gamma radiation (Table 1). The earliest seed germination was observed in control (13.6 days), followed by 40 Gy (25.4 days), 60 Gy (35.6 days), 80 Gy (47.3 days), 100 Gy (50.2 days) and 120 Gy (52.1 days) treatment.Mean germination percentage was the highest in control (92.1%), followed by 40 Gy (76.1%), 60 Gy (52.2%), 80 Gy (32.6%), 100 Gy (16.5%) and 120 Gy (7.4%) treated seeds. Minimum seed germination was observed to be in 120 Gy treatment (Table1). In gamma radiation treatment, per cent seed germination decreased and seedling emergence was delayed with increase in dose of gamma radiations. Similar results were reported by Dhatt *et al.* (2000) and Latado *et al.* (2001) with gamma radiation in citrus. The reduction in germination percentage and delay in germination of kinnow seeds due to gamma ray treatment was also reported by Khokhar (1998). Most of the ill effects of gamma radiation treatment followed immediately after treatment and were manifested in terms of decreased sprouting capacity with increase in the dose (Raghami and Ghazvini 2005).

The mean seedling height and internodal length decreased with increasing dose of gamma radiations (Table 1). The maximum mean seedling height and internode length were observed in the control (10.3 and 2.5 cm) and minimum in 120 Gy (2.4 and 0.7 cm, respectively) treatment. Similarly, Khokhar (1998) observed decrease in mean seedling height and internodal length with increasing gamma radiation doses in citrus. Reduction in plant growth and shoot length was also reported by Waqar *et al.* (1992) in kinnow seedling. Radiation treatments probably induced certain changes at genetic level that ultimately get reflected in the substances that trigger biochemical processes controlling different aspects of the growth. Such substances were identified as auxins, gibberellins, ethylene and abscisic acid, called phytohormones, initiate biochemical reactions and induce changes in chemical composition, there occur changes in chemical patterns which lead to various modifications and variations in plant characters such as height, branching, stem thickness and flowering etc. (Whittwer 1971).

Leaf number, leaf area, number of branches and root length reduced with increase in dose of gamma radiations (Table 1). Reduction in number of leaves and branches in kinnow (Khokhar 1998), and root growth and shoot elongation in grapefruit (Kawamura *et al.* 1989) was also observed with increasing dose of gamma rays. Swaminathan (1965) reported that the radiation was found to cause malfunctioning of various phyto-hormones and cause changes in chemical patterns leading to morphological variations. Radiation treatments also cause quantitative as well as qualitative alteration in the hereditary material. The morphological effects due to radiation have been reported in stem, leaves, branches and even flowers (Lamseejan *et al.* 2000). The variability for number of leaves and number of branches/seedling was also reported in *Lepidiumsativum* (Majeed *et al.* 2010) and *Chrysanthemum* (Datta *et al.* 2005). On the contrary, no such variability was reported by Jawaharlal *et al.* (1992) in acid lime, thereby indicating varietals or genetic specificity of each genotype to radiations.

The number of days taken for germination and per cent germination were significantly affected with increasing doses

Table 1 Effect of gamma irradiation on *in vitro* seed germination and vegetative characteristics of rough lemon seedlings

Treatment	Days taken for germination	Seed germination (%)	Seedling height (cm)	Internodal length (cm)	Leaf number	Leaf area (mm^2)	No. of branches	Root length (cm)	No. of secondary roots
0 Gy	13.6	92.1	10.3	2.5	7.2	203.4	5.2	30.9	8.4
40 Gy	25.4	76.1	7.0	2.1	6.2	197.5	3.6	25.1	6.6
60 Gy	35.6	52.2	5.8	1.7	5.4	124.3	2.6	18.3	5.6
80 Gy	47.3	32.6	4.5	1.0	4.0	94.6	1.8	12.4	4.4
100 Gy	50.2	16.5	3.3	0.9	3.2	68.1	0.0	5.3	2.2
120 Gy	52.1	7.4	2.4	0.7	2.2	0.0	0.0	0.0	0.0
CD (*P*=0.05)	13.9	30.2	2.6	0.7	1.6	70.4	1.8	10.6	2.7

Table 2 Effect of ethyl methane sulphonate on *in vitro* seed germination and vegetative characteristics of rough lemon

Treatment	Days taken for germination	Seed germination (%)	Seedling height (cm)	Internodal length (cm)	Leaf number	Leaf area (mm^2)	No. of branches	Root length (cm)	No. of secondary roots
0.0%	21.7	94.2	10.3	2.5	7.2	203.4	5.2	30.9	8.4
0.2%	30.7	69.9	6.43	1.9	6.2	155.4	4.0	25.2	6.8
0.4%	37.0	64.4	5.1	1.6	5.4	135.1	3.2	19.9	6.2
0.6%	45.3	51.3	4.5	1.2	4.4	115.6	2.3	15.1	5.3
0.8%	49.2	38.2	4.0	0.8	3.2	69.8	1.6	10.2	4.2
1.0%	52.6	22.4	3.0	0.4	2.6	40.3	0.0	4.9	3.4
CD (*P*=0.05)	10.7	22.7	2.3	0.7	1.6	53.0	1.6	8.7	1.6

of ethyl methane sulphonate (Table 2). The number of days taken for germination increased and germination percentage decreased with increase in dose of ethyl methane sulphonate. In control it took 21.7 days for the seed to germinate and 94.2% seed germinated. However, with 1.0% ethyl methane sulphonate concentration number of days taken for seed germination increased to 52.6 days and germination reduced to 22.4%. The delay in seed germination as well as reduction in seed germination percentage with increasing ethyl methane sulphonate doses was also reported by Jawaharlal *et al*. (1992) in acidlime and Khokhar (1998) in kinnow. The sprouting capacity of the seeds fall and they show poor germination after ethyl methane sulphonate treatment was reported due to the effect on cytochrome oxidase content, thus reducing the respiration and hence causing death of the seeds or delayed germination in barley and wheat (Swaminathan *et al.* 1962). The presoaking of seeds was also reported to increase the vulnerability of seeds to ethyl methane sulphonate.The actively dividing phase gets drastically affected so that no germination occur due to alteration of gene (s) or gene complexes (Singh and Singh 1989). Chromosomal aberrations may also occur due to ethyl methane sulphonate treatment which prevents healthy and quicker seed germination.

Seedling height, leaf number, leaf area, number of branches, root length and number of secondary roots reduced with increase in dose of gamma radiations (Table 1). The reduction in mean seedling height because of increasing treatment doses was also reported by Sharma and Sharma (1994) in apple, and Gupta and Sharma (1994) in rice. Khokhar (1998) recorded lower seedling height, internodal length, leaf number and number of branches/seedling in Kinnow mandarin. Mallick *et al*. (1978) suggested that variation in one or more characters might have been due to various mutagenic effects, such as mutation of genes, breaking of tightly linked regions and crossing over within these regions, enhanced recombination, individual or a combination of two or more such effects.The depressing effect of ethyl methane sulphonate on seedling height and other characters in present study might have been due to other physiological damage or due to any of the reasons cited above.

The germination percentage was employed to calculate the mutagen dosage required to kill half of the tested population (LD_{50}) of rough lemon seeds, as seed germination was significantly correlated with both gamma radiation dose (R^2= 0.985) and ethyl methane sulphonate concentration (R^2= 0.978). LD_{50}, the dose required to kill half of the tested population corresponded to 62 Gy for gamma radiation and 0.64% for ethyl methane sulphonate treatment as per forecast analysis done in Microsoft Excel. The LD_{50} value of gamma rays and ethyl methane sulphonate for kinnow seeds was found to be around 10 kR and 0.4%, respectively by Dhatt *et al*. (2000).Waqar *et al*. (1992) reported it to be 10 kR for Kinnow and Hearn (1984) found it to be between 10 and 15 kR for Pineapple sweet orange seeds and 15 kR for Duncan grapefruit seeds and thus concluded that LD_{50} is specific for each variety. Varying value of LD_{50} dose in different citrus cultivars was also reported by Hensz (1971). Similarly, Dhatt*et al.* (2000) reported that LD_{50} dose for kinnow seed with gamma rays slightly less than 10 kR.

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