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Performance of ginger (*Zingiber officinale* Rosc.) to physical and chemical mutagens

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Abstract
A field experiment was conducted to study the influence of physical (gamma rays) and chemical mutagens (EMS) on the sprouting characters and mutation frequency of Ginger local variety Mahim during vM_1 generation. The experiment was conducted at Main garden, Department of Horticulture, Dr. PDKV, Akola, during 2016-17. The ginger rhizomes were irradiated with gamma rays at 0.5, 0.75, 1.00 and 1.25 kR and EMS concentrations at 0.5%, 0.75%, 1.00% and 1.25% along with control respectively. The effect of mutagens on LD50, days to sprouting, sprouting percentage, leaf and rhizome abnormality and mutation frequency were recorded during the vM_1 generation.

Keywords: Gamma rays, EMS, LD50, chlorophyll mutants, vM_1

Introduction
Ginger (*Zingiber officinale* Rosc.) a member of the family Zingiberaceae is an important tropical horticultural perennial herbaceous monocotyledon, (usually grown as annual) is known to human generations as a medicinal and spice crop (Kandiannan *et al*., 1996) [14]. The whole plant is refreshingly aromatic and the underground rhizome, raw or processed, is valued as spice. Ginger is a slender perennial herb, 30-50 cm tall with palmately branched rhizome bearing leafy shoots. The leafy shoot is a pseudostem formed by leaf sheath and bears 8 to 12 distichous leaves. The economic part is the underground rhizome, which is pungent and aromatic. Fresh ginger, dry ginger powder, oleoresin and oil are used in food processing. Ginger has been considered indispensable in the culinary art for flavoring of foods. India is a leading producer of ginger in the world and during 2016-17 the country produced 1081.40 ('000 MT) of the spice from an area of 164.70 ('000 hectares) and 6.5 MT/HA productivity (source: Anon., 2017) [2]. Ginger is cultivated in most of the states in India. The leading states in area wise, production and productivity are Assam, Assam and Gujarat with 18.70 ('000 Ha), 166.50 ('000 MT) and 15.46 MT/Ha respectively (source: Anon., 2017) [2]. The contribution of Maharashtra in ginger production is 8.50 ('000 Ha) area with 125.50 ('000 MT) production and 14.76 MT/HA productivity (Source: Indian Horticulture database, 2017). The major drawbacks of Indian ginger are its high fibre content, high cost of production and susceptibility to various diseases. Hence development of high yielding varieties possessing low fibre content, high volatile oil and oleoresin assumes importance from the point of view of export. However breeding of ginger is seriously handicapped by poor flowering and seed set (Giridharan and Balakrishnan, 1992) [10]. The Most crop improvement programmes of this species are confined to evaluation and selection of naturally occurring variations. Mutation induction has become a proven way of creating variation within a crop variety. It offers the possibility of inducing desired attributes that either cannot be expressed in nature or have been lost during evolution (Novak and Brunner, 1992) [19]. In non-seed setting vegetatively propagated crops like ginger mutation breeding is one of the methods of creating genetic variability which could be used for subsequent improvement.

Materials and Methods
The ginger rhizomes were irradiated with gamma rays at 0.5, 0.75, 1.00 and 1.25 kr in the Bhabha Atomic Research Centre (BARC), Trombay, Mumbai, Maharashtra, India. Rhizomes were presoaked in EMS concentrations at 0.5%, 0.75%, 1.00% and 1.25% for 4 h. The treated rhizomes were immediately sown in poly bags along with control and later transplanted in main field at 60 days after sowing. The experiment was laid out in Randomized Block Design

(RBD) with three replications. The days to sprouting, sprouting percentage, leaf and rhizome abnormality and mutation frequency was recorded and worked out. The sprouting (%), leaf and rhizome abnormality (%) in each treatment was calculated as no. of rhizomes sprouted and showed abnormality to the total number of rhizomes planted, whereas mutation frequency was calculated as the no. of mutants observed to the total population and expressed in %.

$$\text{Sprouting percentage} = \frac{\text{No. of rhizomes sprouted}}{\text{Total no. of rhizomes planted}} \times 100$$

$$\text{Mutation frequency (\%)} = \frac{\text{Number of mutants}}{\text{Total number of plants scored}} \times 100$$

Result and Discussion

The observations were recorded on various sprouting parameters viz., days to sprouting and sprouting percentage in ginger, and the results were summarized below.

1. LD50

Lethal dose 50 (LD50) can be defined as the mutagen doses/concentration and conditions that contributes to 50% lethality (Out of the total number of seeds). Determination of LD50 is necessary to produce a high frequency of desirable mutations (Hohmann *et al*., 2005; Arisha *et al*., 2014; Arisha *et al*., 2015) [12, 3, 4]. The LD50 obtained by treating ginger variety "Mahim" to different doses of gamma rays (0.5 kR, 0.75 kR, 1.00 kR and 1.25 kR) and Ethyl methane sulphonate (0.5 %, 0.75 %, 1.00 % and 1.25 % EMS) along with Control. Based on the population survival after treatment, it was concluded that the LD50 for gamma rays ranged between 0.75 kR - 1.00 kR and for EMS it was obtained at 1.00 % EMS. Similar results for the LD50 value as 0.5 - 1.5 kR gamma rays and 0.9 - 1.5 % for EMS were obtained by Mohanty and Panda (1988) [17] and 1.0 and 1.25 kR gamma rays based on the surviving plants by Jayachandran and Mohankumar (1992) [13] and Nwachukwu *et al*., (1994) [20] reported that LD50 obtained at 8.75 Gy in ginger.

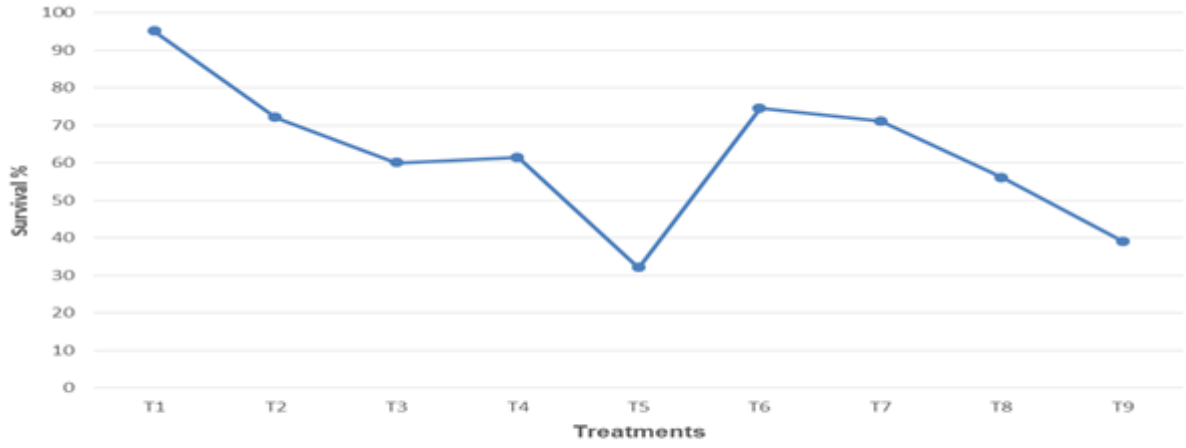

Fig. 1 Optimization of LD50 Value of ginger in gamma rays and EMS

2. Days to sprouting

Among the different doses and concentrations of gamma rays and EMS along with control (untreated) treatments, the significantly minimum days to sprouting was recorded in treatment T_1 (12.27 days), followed by the treatments T_2 (16.73 days) and the maximum days to sprouting was recorded in treatment T_5 (28.93 days). From the data recorded, it is clearly observed that the treatment of the rhizomes with mutagens resulted in delayed sprouting. The days to sprouting increased as the doses and concentrations of gamma rays and EMS increased. Delay in sprouting may be due to the level of chromosomal damage caused by increasing doses of mutagens, reduce growth regulators such as cytokines by breaking them down or not synthesizing, thereby increasing plant sensitivity, damage of cell constituents, alteration of enzyme activity or delay or inhibition of physiological and biological processes or may also be due to the seeds absorbing the mutagen, which subsequently reaches the meristemic region and affects the germ cell. Low levels of mutagens also induce growth stimulation signals by increasing the antioxidative ability of cells or by changing the hormonal signaling in plants and in the early stages of seed germination triggers the activation of RNA or protein synthesis. Similar research findings were also reported by Giridharan and Balakrishnan (1992) [10] in ginger; Nwachukwu *et al*., (1994) [20] in ginger and Asare and Akama (2014) [5] in sweet potato.

3. Sprouting percentage

The data recorded on the different doses and concentrations of gamma rays and EMS along with control (untreated) treatments, the significantly maximum sprouting percentage was recorded in treatment T_1 (95.03 %), followed by the treatments T_6 (87.31 %) whereas, the minimum sprouting percentage was recorded in treatment T_5 (41.33 %). From the observations recorded, it was observed that the sprouting percentage decreased by the treating the material with both gamma rays and EMS at 30 DAS. The increase in the doses and concentrations of gamma rays and EMS, decreased the sprouting percentage Higher sprouting percentage at lower doses may be due to the resistant nature of the plant material to a certain doses (gamma rays) and concentrations (EMS) and also due to the break in the dormancy, which resulted in stimulation of sprouting. The increasing doses of mutagens are injurious to the plant cell and ultimately interfere with the growth of plants. The decrease in sprouting may be due to lethality caused in sprouts, physiological injuries and the gamma ray reaction with the nucleic acid like DNA by

alkylating their phosphate group. The hydrolytic products also damage the cell membrane and other cell constituents at molecular level leading to breaks, physiological injuries and ultimately stopping the metabolic activity of the cells, change in metabolic condition of cells, delay in the initiation of metabolism, drop in auxin level, delay in the one set of mitosis resulting in uniform delay in mitotic activity and chromosomal aberration induced enzyme activity or defective enzyme production such as catalase, lipase and hormonal activity, seedling growth, and ATP and DNA synthesis resulting in the decrease of the sprouting per cent. The similar results like decrease in the sprouting with an increase in doses were also reported by Choudhary and Dnyansagar (1980) [7] in garlic, Raju *et al*., (1980) [23] in ginger, turmeric and mango-ginger, Giridharan (1984) in ginger, Kataria and Singh (1989) [15] in onion, Malani *et al*., (1993) [16] in okra, Amjad and Anjum (2002) [1] in onion, Omar *et al*., (2008) [21] in chilli, Devi and Mullainathan (2011) [8] in chilli and Baghery *et al*., (2015) [6] in okra in onion.

Table 1: Effect of gamma rays and EMS on days to sprouting, sprouting (%), leaf abnormality (%), rhizome abnormality (%) and mutation frequency (%) in ginger in vM_1 generation

Treatments	Days to sprouting (no.)	Sprouting (%)	Leaf abnormality (%)	Rhizome abnormality (%)	Mutation frequency (%)
T_1 - Control	12.27	95.03 (77.12)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Physical mutagen (Gamma rays)					
T_2 - 0.5 kR	16.73	82.00 (64.90)	4.67 (12.48)	8.00 (16.43)	23.33 (28.88)
T_3 - 0.75 kR	24.33	75.33 (60.22)	0.67 (4.70)	9.33 (17.79)	12.00 (20.27)
T_4 - 1.00 kR	27.93	67.33 (55.14)	1.33 (6.62)	6.00 (14.18)	8.67 (17.12)
T_5 - 1.25 kR	28.93	41.33 (40.01)	2.00 (8.13)	6.67 (14.97)	9.33 (17.79)
Chemical mutagen (Ethyl methane sulphonate)					
T_6 - 0.5 % EMS	16.87	87.31 (69.13)	7.99 (16.42)	5.00 (12.92)	15.00 (22.79)
T_7 - 0.75 % EMS	20.20	75.01 (60.01)	12.98 (21.12)	8.00 (16.43)	29.94 (33.17)
T_8 - 1.00 % EMS	26.93	70.94 (57.38)	14.97 (22.76)	9.00 (17.46)	27.98 (31.94)
T_9 - 1.25 % EMS	28.07	63.04 (52.56)	16.99 (24.34)	10.01 (18.44)	31.99 (34.44)
F test	Sig	Sig	Sig	Sig	Sig
SE(m)±	0.39	2.76	1.24	1.43	1.77
CD at 5%	1.19	7.57	3.76	1.09	5.34

(Figures in the parentheses are arcsine transformed values)

4. Leaf abnormality (%)

The different doses and concentrations of gamma rays and EMS along with control (untreated), caused the leaf abnormality (%) ranged from 0.00-16.99% in case of ginger. The significantly minimum leaf abnormality (%) was recorded in treatment T_1 (0.00 %), followed by the treatments T_3 (0.67 %) and the significantly maximum leaf abnormality (%) was recorded in treatment T_9 (16.99 %) followed by the treatments T_8 (14.97 %). The gamma rays and EMS treated population showed defective plants having abnormal plants with unopened leaves, leaves showing white stripes on affected areas, pale green leaves, yellow cotyledonary leaves or white lesions on the leaves and variegation. Different components of photosynthesis altogether such as pigment protein complexes which play a role in absorbing the light, enzymes reduced for the carbon reduction cycle and electron transport carriers. This photosynthetic complex responsible for performing various activities is altered by the radiations. Ionizing radiations decrease the capabilities of the photosynthetic apparatus by damaging the photosystem. The mutagens cause ultrastructural changes in the irradiated plant cell, which shows that chloroplasts are sensitive to gamma rays as compared to other organelles present in the plant cell. Plastids were also found to be affected as senescence was inhibited and due to differentiation into the agranal stage. Variegations in the leaves might have been produced by nuclear or plastid mutations. They were of the view that spontaneous or induced plastid mutations produced a variety of phenotypes such as cream, white and various shades of pale green colour. This chimeric pattern of variegated leaves depends on the occurrence of mutations in different growing points. (Priya *et al*., 2014) [22].

Gamma rays are categorized in ionizing radiation because these radiations produce free radicals in the cell when they interact with atoms or molecules. These free radicals damage the cell, but sometimes modify the cells and components. Damage or modification of the cells and components depends upon the level of radiation. These radiations cause changes in the physiology, morphology, anatomy and biochemistry of the plants. The effect of these radiations is dose dependent, as these rays stimulate growth in plants at low dose Therefore, these radiations are important in modifying the plant genome for crop improvement. The abnormalities may be due to the chromosomal aberrations, metabolic hinderance of enzyme activity, disturbances in the growth and development of rhizomes, disturbances in the production and distribution of growth substances. The data recorded showed that the leaf abnormality increased with increased concentration of EMS rather than the gamma rays. The effect of gamma rays is not so prominent as compared to EMS in creation of leaf abnormality. Similar findings were reported by Mohanty and Panda (1988) [17] in ginger, reported EMS to be potent mutagen for morphological mutations, Kataria and Singh (1989) [15] in onion, Usha Nandini Devi (2004) [24] reported varied chlorophyll mutants with increased dose of gamma rays, Devi and Mullainathan (2011) [8] in chilli and Girija and Dhanavel (2013) [11] in cowpea,

5. Rhizome Abnormality (%)

The different doses and concentrations of gamma rays and EMS along with control (untreated), caused the rhizome abnormality (%) ranged from 0.00 -10.01% in ginger. The significantly minimum rhizome abnormality (%) was recorded in treatment T_1 (0.00 %), followed by the treatments T_6 (5.00 %) and the significantly maximum rhizome abnormality (%) was recorded in treatment T_9 (10.01 %) followed by the treatments T_3 (9.33 %) and T_8 (9.00 %). The data recorded showed that rhizome abnormality increased with increased concentration of EMS rather than the gamma rays. The type of abnormalities caused in rhizome may be irregular shape of rhizomes, fingers of rhizomes, uneven development of rhizomes, compact rhizomes, restricted

intermodal elongation of rhizomes etc. The effect of gamma rays is not so prominent as compared to EMS in creation of rhizome abnormality. Gamma rays are categorized in ionizing radiation because these radiations produce free radicals in the cell when they interact with atoms or molecules. These free radicals damage the cell, but sometimes modify the cells and components. Damage or modification of the cells and components depends upon the level of radiation. These radiations cause changes in the physiology, morphology, anatomy, and biochemistry of the plants. The effect of these radiations is dose dependent, as these rays stimulate growth in plants at low dose Therefore, these radiations are important in modifying the plant genome for crop improvement. The abnormalities may be due to the chromosomal aberrations, metabolic hinderance of enzyme activity, disturbances in the growth and development of rhizomes, disturbances in the production and distribution of growth substances.

6. Mutation frequency (%)

Among the different doses and concentrations of gamma rays and EMS along with control (untreated), the mutation frequency (%) ranged from 0.00 (0.00) - 31.99 %. The significantly minimum mutation frequency (%) was recorded in treatment T_1 (0.00), followed by the treatments T_4 (8.67 %) and T_5 (9.33 %) and the significantly maximum mutation frequency (%) was recorded in treatment T_9 (31.99 %) followed by the treatments T_7 (29.94 %). The recorded data on mutation frequency (%) showed that increased doses of gamma rays reduced mutation frequency (%) whereas increased concentration of EMS increased mutation frequency (%) with maximum at highest dose of EMS. It may be due to more chromosomal aberrations, DNA mutations, injury to the growing points, physiological, anatomical damage and disturbances in the production and distribution of growth substances by the treatment of biological material with heavy doses of gamma rays and EMS. The results are in accordance with the Mohanty and Panda (2008) in ginger and Neopaney (1994) [18] in ginger.

Conclusion

The results of the present study indicate that both the gamma rays and EMS found be to effective mutagens for mutagenesis of ginger.

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