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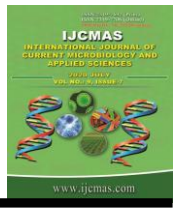

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Assessment of Mutagenicity Induced by Gamma Rays and EMS in M1 Generation of Chilli (*Capsicum annuum* L.)

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A B S T R A C T

To assess the effect of gamma rays and EMS (Ethyl methane sulfonate) application on chilli var. Kashi Anmol, an experiment was conducted during winters of 2017-18 at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. The experimental design was randomized block design and replicated thrice. Treatments include one control, five doses of gamma rays (10 kR, 20 kR, 30 kR, 40 kR and 50 kR), five doses of EMS (10 mM, 20 mM, 30 mM, 40 mM and 50 mM) and their combinations (gamma rays 10 kR + 20 mM EMS, gamma rays 20 kR + 20 mM EMS, gamma rays 30 kR + 20 mM EMS, gamma rays 40 kR + 20 mM EMS and gamma rays 50 kR + 20 mM EMS). There was substantial reduction in seed germination percentage, seedling height, pollen fertility, ovule fertility and plant survival with an increase in doses of gamma rays, EMS and their combination. The EMS was observed more effective mutagen than gamma rays and their combinations even more.

Introduction

Chilli (*Capsicum* spp.) is one of the most important and the largest produced spice in India. Chillies, both fresh and dried are used as condiments and culinary supplements for their pungency and characteristics pleasant flavours. Among the five cultivated genus of the *Capsicum*, *C. annuum* is most widely cultivated in India and an important commercial spice and vegetable crop for small and marginal farmers (Reddy *et al.*, 2014). At present people are much concerned about the fruit quality and yield. Therefore, attention is being paid for development of genotypes having high yield potential with desirable fruit quality characters in a short period of time (Meghvansi *et al.*, 2014).

It is a well-known fact that genetic variations have practical implication in crop improvement. Induced mutation is a method by which novelty can be created in already well-established cultivars and it also creates

genetic variation (Tiwari *et al.*, 2018). Many physical and chemical mutagens have been used for induction of useful mutations in number of crops. Mutation is the ultimate source of all genetic variation and provides the raw material for evolution (Saba and Mirza, 2002). Induced mutations can rapidly create variability in quantitatively and qualitatively inherited traits in crops. Gamma rays (γ) are ionizing radiations and interact with atoms and molecules to produce free radicals in cells. The advantages of ionizing radiations as mutagens are accurate dosimetry, reasonable reproducibility and uniform penetration of multicellular system particularly by gamma rays (Jain, 2005). EMS is a chemical mutagen and used frequently and abundantly in plant systems as it causes a high frequency of nucleotide and substitution variations. Both mutagens are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues. Kashi Anmol is one of the high yielding varieties of chilli and moderately resistant to anthracnose, die back and *Cerscopora* leaf spot under field conditions. Keeping this in view the present investigation was carried out to assess the effect of gamma rays, EMS and their combinations on chilli var. Kashi Anmol.

Materials and Methods

The present investigation was carried out during 2017-18. Uniform and healthy seeds of chilli var. Kashi Anmol were selected for mutagenic treatment. 500 seeds of var. Kashi Anmol per treatment were irradiated with five different dosses of gamma rays *viz.*, 10 kR, 20 kR, 30 kR, 40 kR and 50 kR at NBRI (National Botanical Research Institute), Lucknow. For treatment with EMS, seeds of each treatment were first soaked in distilled water for 12 hours at room temperature and consequently dried with the help of blotting paper and finally emerged in ethyl methane sulphonate (EMS) at five different concentration i.e., 10 mM, 20 mM, 30 mM, 40 mM and 50 mM in phosphate buffer (pH 7.0) for 06 hours and then washed thoroughly in running tap water to eliminate the residual effect of the chemical. 500 seeds already eradiated with five different dosses (10kR, 20kR, 30kR,40kR and 50kR) of gamma rays, were treated with 20 mM EMS solution, followed by washing in running tap water in the similar manner as described above for combination treatment of gamma rays and EMS treatment. A small portion of treated seeds along with control were used for observation on germination test and seedling height reduction in the laboratory, whereas the major portion of seeds were sown in nursery bed and further transplanted to main experimental blocks to raise M1 generation. The experimental design was randomized block design and replicated thrice at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Results and Discussion

The induced effects in M1 generation caused by physical and chemical mutagens and their combination towards physiological damage and chromosomal mutations in the biological material could be measured quantitatively by the degree of reduction in germination, plant survival, seedling growth and fertility percent (pollen and ovule) and the frequency of chromosomal aberrations and chimeras (Gaul *et al.*, 1972).

Seed germination (%)

A reduction in germination per cent was observed at higher doses of gamma rays, EMS and their combined treatments. However, germination was less affected by lower concentration of EMS. The reduction in

seed germination per cent was due to the interruption of physiological and biological processes indispensable for seed germination including enzymatic activity (Kurobane *et al.*, 1979). The seed germination as per cent of control was maximum (97.95 %) and minimum (79.59%) for seeds treated with gamma rays 10 kR and 50 kR respectively. Whereas for EMS, it was maximum (93.87%) and minimum (81.63%) for seeds treated with 10mM and 50mM, respectively. However for the combination effect of gamma rays and EMS maximum (93.87%) and minimum (77.55%) seed germination was observed for seed treated with 10kR of gamma rays + 20mM EMS and 50kR of gamma rays + 20 mM of EMS, respectively.

These results are corroborated with the results of Singh *et al.*, (2006), Tomlekova *et al.*, (2007), Omar *et al.*, (2008), Sri Devi and Mullainathan (2012) , Sri Devi and Selvakumar (2013) and Gaur *et al.*, (2014) in chilli who reported gradual decrease in germination percent with increasing doses of mutagens.

Seedling height

There was a gradual decrease observed in seedling height with the increase in dosses of gamma rays, EMS and their combined treatments in chilli, whereas it was found less effective when treated with a lower concentration of EMS. The reduced growth of seedling has been explained on the basis of auxins destruction, changes in ascorbic acid content and physiochemical and biochemical disturbances (Usuf and Nair, 1974). Treatments with gamma rays, EMS and their combination resulted in slight to severe reduction in seedling height. The higher the doses of mutagens, the greater was the reduction in seedling height. The lowest seedling height reduction 9.37 as per cent of control was observed in 10kR gamma rays and highest seedling height reduction 78.12 as per cent of control in 50kR+ 20 mM gamma ray and EMS combination. The seedlings height reduction ranged from9.37 -71.87, 25.00 -50.00 and 31.25 – 78.12 in gamma rays, EMS and their combinations, respectively. These findings are in line of earlier workers *viz.,* Tomlekova *et al.,* (2007), Omar *et al.,* (2008), Sri Devi and Mullainathan (2012) and Sri Devi and Selvakumar (2013) and Gaur *et al.,* (2014) in chilli.

Plant survival at maturity

The survival of M1 plants decreased considerably with the mutagenic treatments. However, no dose dependent relationship was noticed. Gaul (1964) considered that reduction in survival percent of M1 plants could be attributed to chromosomal and point mutations may be attributed to the cause of reduction in germination and plant survival percent due to mutagenic treatments in M1 generations (Gaul *et al.*, 1972).

The survival of plant till maturity depends upon the normal physiochemical balance of cell metabolism. The mutagens are capable of creating chromosomal and extra chromosomal abnormalities. These abnormalities have lethal effects at different stages of growth. Physiological imbalances or different types of chromosomal aberrations might be the prime cause of reduction survival percentage in M1 plants. In general the plant survival decreased with increase in the doses of gamma rays, EMS and combined treatment of both the mutagens. Maximum plant survival per cent was observed for control (96.76 %) followed by 10 KR gamma rays +20 EMS (95.00%) and 10 kR gamma rays (93.33%). Plant survival as per cent of control ranged from 96.54 – 86.20% for gamma rays, 94.82 – 93.10% for EMS and 98.27 – 82.75% for the combination of gamma rays and EMS.

Table.1 Seed germination, seedling height reduction, pollen fertility, ovule fertility and plant Survival (as percentage of control) of chilli variety 'Kashi Anmol' treated with gamma rays, EMS individually and their combination in M1 generation

Mutagenic Treatments	Percent Seed Germination	Germination as per cent of control	Mean Seedling height (cm)	Seedling height reduction as per cent of control	Mean pollen fertility %	Pollen Fertility as per cent of control	Mean ovule fertility %	Ovule Fertility as per cent of control	Per cent plant survived	Survival as per cent of control
Gamma Rays (kR)										
10	96	97.95	2.9	9.37	89.28	90.54	71.80	92.74	93.33	**96.54**
20	94	95.91	2.7	15.62	84.77	85.97	68.35	88.28	90.00	**93.10**
30	92	93.87	1.4	56.25	83.20	84.38	65.22	84.24	86.67	**89.65**
40	88	89.79	1.0	68.75	78.12	79.22	61.30	79.17	85.00	**87.92**
50	78	79.59	0.9	71.87	75.80	76.87	58.60	75.69	83.33	**86.20**
EMS(mM)										
10	92	93.87	2.4	25.00	86.54	87.76	67.20	86.79	91.67	**94.82**
20	90	91.83	2.2	31.25	82.42	83.59	64.66	83.51	88.33	**91.37**
30	88	89.79	1.8	43.75	79.20	80.32	61.35	79.24	88.33	**91.37**
40	84	85.71	1.9	40.62	77.12	78.21	57.10	73.75	86.6	**89.65**
50	80	81.63	1.6	50.00	75.60	76.67	55.80	72.07	90.00	**93.10**
Gamma rays(kR)+ 20mM EMS										
10	92	93.87	2.2	31.25	84.77	85.97	62.40	80.59	95.00	**98.27**
20	88	89.79	2.0	37.50	79.20	80.32	58.30	75.30	91.67	**94.82**
30	84	85.71	1.2	62.50	74.32	75.37	54.10	69.87	90.00	**93.10**
40	82	83.67	0.8	75.00	71.60	72.61	51.60	66.84	86.00	**89.65**
50	76	77.55	0.7	78.12	68.22	69.18	48.22	62.28	80.00	**82.75**
Control	**98**	**100**	**3.2**	**100**	**98.60**	**100**	**77.42**	**100**	**96.76**	**100**

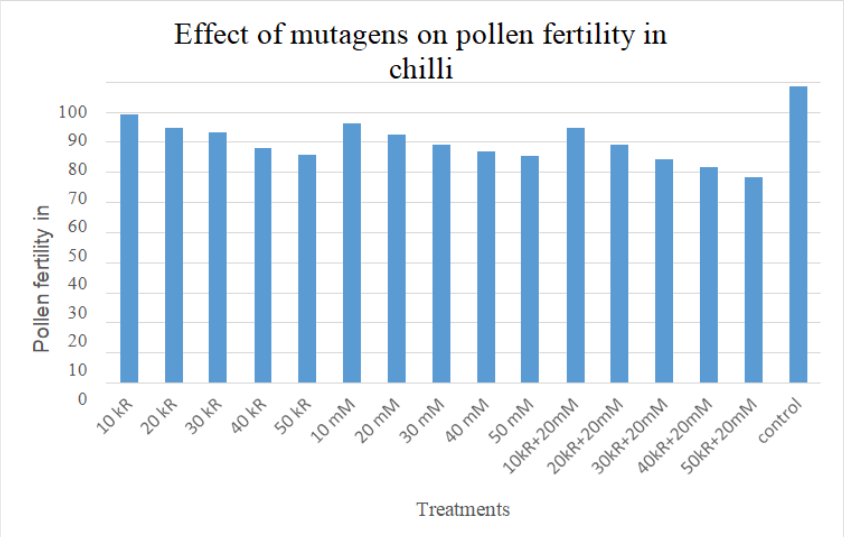

Fig.1 Pollen fertility (%)

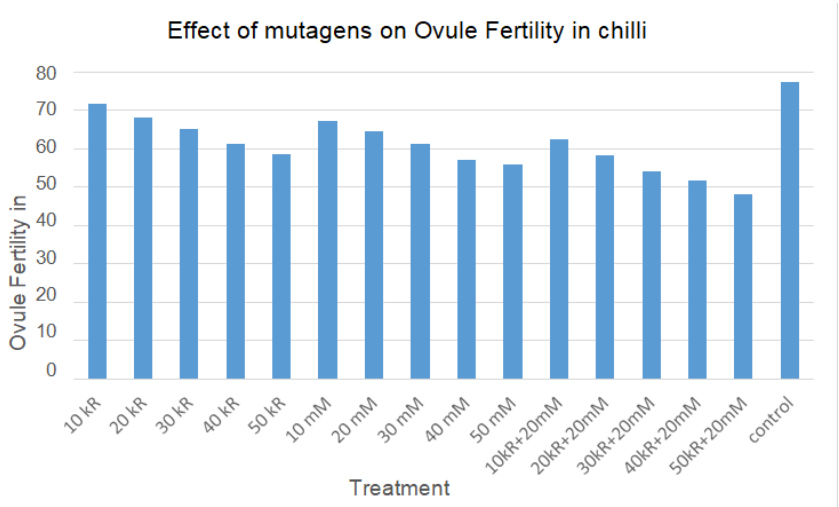

Fig.2 Ovule fertility (%)

Pollen and ovule sterility percentage

The study revealed that an increase in doses of Gamma rays, EMS and their combinations substantially decreased pollen fertility and ovule fertility. The structural changes in chromosomes are the possible cause of sterility.

The higher degree of pollen sterility was reported to be linked with meiotic abnormalities (Muthusamy and Jayabalan, 2002; Khan and Wani, 2005), because meiosis was more prone to conceivable type of turbulences (Khan and Goyal, 2009). According to Sudhakaran (1971) and Konzak *et al.,* (1961) induction of pollen sterility was due to chromosomal abnormalities produced by mutagens. It was apparent from the data that the higher doses of mutagens were more effective in reducing the pollen fertility.

Maximum pollen fertility (90.54%) as per cent of control was observed in gamma ray 10 kR whereas minimum (69.18%) pollen fertility as per cent of control was observed in combination of 50kR gamma ray + 20 mM EMS.

The range of pollen fertility as per cent of control for gamma rays, EMS and their combinations were (90.54 to 76.87%), (87.76 to 76.67%) and (85.97 to 69.18%), respectively.

The higher doses of gamma rays, EMS and their combination were more effective in reducing the ovule fertility with maximum reduction of 62.28% being in gamma ray and EMS combination of 50kR + 20mM EMS, whereas it was minimum 92.74% in treatment with gamma ray of 10kR. In general the higher doses of the mutagens, the lower was the ovule fertility as percent of control. The ovule fertility as percent of control varied from 92.74 to 75.69 for gamma rays, 86.79 to 72.07 for EMS and 80.59 to 62.28 for the combinations of gamma rays and EMS. The findings were in accordance to the observation of Gaur *et al., (2014)* in chilli.

Thus the study revealed that there was substantial reduction in seed germination percentage, seedling height, pollen fertility, ovule fertility and plant survival with an increase in doses of gamma rays, EMS and their combination. The EMS was more effective than gamma rays and their combinations even more.

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