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RESEARCH ARTICLE

Frequency and spectrum of chlorophyll mutation in chilli (*Capsicum annuum* L.)

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SUMMARY

A comparison of the effect of gamma-rays ethyl methane sulfonate (EMS) and their combination for induced mutagenesis in two chilli varieties Kashi Anmol (KA-2) and VR-338 (Kashi Gaurav). 1000 chilli seeds each were irradiated with 10,15, 20, and 25 kR doses of gamma-rays treated, with 0.02, 0.03 0.04 and 0.05 M concentration of ethyl methane sulfonate and another 1000 seeds used for combination treatments were treated with all doses of gamma-rays followed by 0.03M EMS. The M_1 generation was raised in protected nursery at Horticulture Research Farm, BHU, Varanasi during July 2011. Chlorophyll mutations were used to evaluate the mutagenic effeciency of various mutagens. The spectrum of chlorophyll mutants Albino, Chlorina, Viridis Xantha and Dark Xantha were most frequently observed in M_2 generation. Gamma rays induced higher proportions of chlorophyll mutants than EMS and their combination. However, frequencies of viable chlorophyll mutants were observed in higher EMS and gamma-rays treatments than with their combination. Treatment with 15 kR gamma-rays was more effective in inducing chlorophyll mutations with highest frequency. Mutagenic effect generally increased with increasing dose of and gamma-rays EMS and their combination.

Key Words : *Capsicum annuum,* Induced mutation, EMS, Gamma-rays, Chlorophyll

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Chilli peppers have been a part of the human diet in the Americas since at least 7500 BC. It originated in the Americas (Guatemala). Chilli (*Capsicum annuum* L.) is one of the most cultivated vegetable spice crops in tropical and subtropical climates. India is the largest consumer and exporter of chilli in the international markets and exports dry chilli, chilli powder and oleoresins (biological colouring agent) to over 90 countries. The production of chilli in India is dominated by Andhra Pradesh which bestows 53 per cent to the total area production. It is grown in several parts of India has a larger area; its productivity is low when compared to other countries. Hence, there is an urgent need to produced and identify new varieties combining high level of disease resistance, besides increased yield and capsaicin content in chilli. Mutagenesis is one of the most critical steps for genetic studies as well as selective breeding. Mutations are the tools and being used to study the nature and basis of plant growth and development, thereby producing raw materials for genetic improvement of crops. Induced mutations can rapidly create variability in quantitatively and qualitatively inherited traits in crops.Various mutagenic agents are used to induce favourable mutations at high frequency that include ionizing radiation and chemical mutagens. Successful mutant isolation largely relies on the use of efficient mutagens. In plant research, a chemical mutagen, ethyl methane sulfonate (EMS) produces single base substitutions with different mutation spectra. Chemo-mutagens induce a broad variation of morphological and yield structure parameters in comparison to normal plants. The present study was undertaken to gather information on the response of chilli genotype to varying doses of gamma-rays irradiation, chemical mutagens (EMS) and their

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combination and determine the type and frequency of mutations.

MATERIALS AND METHODS

Chilli variety Kashi Anmol (KA-2) and VR-338 (Kashi Gaurav) was irradiated with 10, 15, 20, and 25 kR with a Co_{60} gamma cell, at National Botanical Research Institute Lucknow, India. Two thousands seed of each variety were used for each dose of gamma-rays irradiation. Half of these irradiated seeds were used for combination treatments with 0.03M EMS. 4000 seeds of each variety were soaked in double distilled water for 12 hrs. The soaked seeds were treated in separate sets (1000 seeds in each sets) with 0.02, 0.03, 0.04 and 0.05 M EMS concentrations. The graded solution of EMS was prepared freshly in phosphate (buffer pH 7.0). The seeds were completely submerged in treatment media for 6 hrs. The treated seeds were washed thoroughly in running tap water. All mutagen treated seeds along with control were sown in the poly house and after 25-30 days the nursery were transplanted in Randomized Block Design in three replications, at Agriculture Research Farm Institute of Agricultural Sciences, B.H.U. to raise M_1 generation. All the recommended cultural practices were carried out during the plant growth period.

All surviving M_1 plants were selfed and harvested to form M_2 generations. From each treatment in M_1 , a total of 100 seeds in twelve sets with one control (untreated) of each variety were placed on moistened germination paper to determine lethality on the basis of seed germination were noted from emergence until 2-3 weeks after germination. Chlorophyll mutations, as described by Gustaffson (1940) were scored throughout the plant growing period.

RESULTS AND DISCUSSION

Chlorophyll mutation were recorded in the 10-20 days old seedling . It provides one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in various crops by several workers like pulse crop including Gautam *et al*. (1992). In chilli, during M_2 generation various individual obtain from M_1 generation bearing different types of colour appeared in seedling stage of M_2 progenies of each variety of chilli. Colour is affected by present of amount chlorophyll in leaves. It is known as chlorophyll mutations and it is also known as macro mutations. Among the M_2 seedling gamma-rays EMS and their combination induce five different types of chlorophyll mutations within each variety of chilli, in which three types of chlorophyll mutations (Albino, Xantha, Dark Xantha) were lethal, and two types (Viridis, Chlorina) were viable.

The reported chlorophyll mutant types were (Plate 1-10) :

Albino:

White, lethal, neither carotenoids nor chlorophyll were formed.

Xantha :

Yellow to yellowish white, lethal, carotenoids present but chlorophyll absent

Dark Xantha :

Dark yellow, It is lethal

Viridis :

Uniform light yellow green colour of leaves, It is viable.

Chlorina :

Uniform green colour with white on tips, It is also viable.

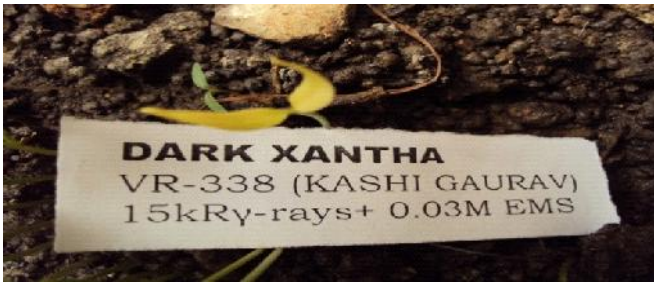
High frequencies of chlorophyll mutation were found in gamma-rays doses than all the doses of EMS their combination in both variety of chilli (Table 1). Highest frequency of chlorophyll mutation (2.09%) was found in 15 and 25kR dose of gamma-rays in Kashi Anmol and lowest frequency of chlorophyll mutation (0.81%) with the treatment of 10kR of gamma-rays in Kashi Anmol . But gamma ray is the more effective for inducing chlorophyll mutation in comparison to EMS and their combination. Five types of chlorophyll mutation (Albino, Xantha, Dark Xantha, Viridis, Chlorina) were found in 0.02 and 0.03M dose of EMS. The Highest frequency of Albino (1.09%) chlorophyll mutants was recorded in 15kR of gamma-rays in Kashi Anmol, Lowest frequency of Albino (0.08%) chlorophyll mutants was recorded in combination of 25kR gamma-rays and 0.03M EMS. Highest frequency of Xantha (1.63%) chlorophyll mutants was found in 0.05M treatment of EMS in Kashi Anmol and lowest frequency of Xantha (0.09%) chlorophyll mutants was found in 0.02M concentration of EMS in VR-338. Dark Xantha was recorded higher frequency (0.84%) in 25kR of gamma-rays in Kashi Anmol and lowest frequency (0.08%) was recorded in 25kR gamma-rays+0.03M EMS treatment of combination in VR-338. Highest frequency of Viridis (0.58) and Chlorina (0.37) chlorophyll mutants was found in 0.04M dose of EMS and 20kR dose of gamma-rays respectively in VR-338 and lowest frequency of Viridis (0.09%) was recorded in 10kR +0.03 combination of gamma-rays and EMS in VR-338. Total frequency of lethal types of chlorophyll mutation (15.06%) were recorded in Kashi Anmol and (10.19%) were recorded in VR-338, respectively. Total frequency of viable types of chlorophyll mutation 3.18 per cent were recorded in Kashi Anmol and 2.73 per cent were recorded in VR-338, respectively. The viable types of chlorophyll mutation were remarkably less frequent than the lethal in both variety of chilli.

Xantha type of chlorophyll mutation recorded the highest frequency fallowed by Dark Xantha, Albino, Viridis, and Chlorina ranked in descending order within the treated progenies of both variety. The frequency of chlorophyll mutation recoverable in M_2 generation may taken as a good indicator for the frequency of mutation with less expression and it could be the most reliable measure for evaluating the


ALBINA
KA-2 γ-rays 15 kR


ALBINA
VR-338 (KASHI GAURAV)
γ-rays 25 Kr


DARK XANTHA
KA-2 (0.03 M EMS)


DARK XANTHA
VR-338 (KASHI GAURAV)
15kRγ-rays+ 0.03M EMS


VIRIDIS
KA-2 γ-rays 15 Kr


VIRIDIS
VR-338 (KASHI GAURAV)
γ-rays 20 Kr


XANTHA
KA-2 γ-rays 15 Kr


XANTHA
VR-338 (KASHI GAURAV)
15kRγ-rays+ 0.03M EMS


CHLORINA
KA-2 γ-rays 15 Kr


CHLORINA
VR-338 (KASHI GAURAV)
γ-rays 20 Kr

Table1: Effect of different doses of Gamma-rays EMS and their combination on frequency and spectrum of chlorophyll mutation in M_2 generation

KASHI ANMOL (KA-2)

		Chlorophyl mutants						Frequency and spectrum of chlorophyll mutants/1000 M_2 plant									
								Non viable mutants						Viable mutants			
	Dose	Number of M_2 plant	Number	Frequency /1000M_2 plant	Total % of Chlorl. muts.	Total non viable muts.	Total viable muts.	Albino	%	Xantha	%	Dark Xantha	%	Viridis	%	Chlorina	%
Control		1017.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gamma-rays	10.00	1110.00	9.00	8.11	0.81	0.27	0.54	--	--	1.80	0.18	0.90	0.09	5.41	0.54	--	--
(kR)	15.00	1098.00	23.00	20.95	2.09	1.64	0.27	10.93	1.09	3.64	0.36	1.82	0.18	--	--	2.73	0.27
	20.00	1005.00	19.00	18.91	1.89	1.49	0.40	4.98	0.50	5.97	0.60	3.98	0.40	3.98	0.40	--	--
	25.00	1196.00	25.00	20.90	2.09	2.01	0.08	--	--	11.71	1.17	8.36	0.84	--	--	0.84	0.08
EMS (M)	0.02	1156.00	14.00	12.11	1.21	1.04	0.17	--	--	6.06	0.61	4.33	0.43	1.73	0.17	--	--
	0.03	1230.00	17.00	13.82	1.38	0.98	0.40	2.44	0.24	4.07	0.41	3.25	0.33	2.44	0.24	1.63	0.16
	0.04	1266.00	23.00	18.17	1.82	1.82	--	3.16	0.32	15.01	1.50	--	--	--	--	--	--
	0.05	1285.00	25.00	19.46	1.95	1.95	0.31	--	--	16.34	1.63	3.11	0.31	--	--	--	--
Gamma-rays +	10.00	1220.00	11.00	9.02	0.90	0.74	0.16	1.64	0.16	5.74	0.57	--	--	1.64	0.16	--	--
0.03M EMS	15.00	1299.00	17.00	13.09	1.31	0.92	0.39	2.31	0.23	--	--	6.93	0.69	3.08	0.31	0.77	0.08
	20.00	1317.00	17.00	12.91	1.29	1.06	0.23	--	--	6.83	0.68	3.80	0.38	2.28	0.23	--	--
	25.00	1300.00	18.00	13.85	1.38	1.15	0.23	0.77	0.08	8.46	0.85	2.31	0.23	1.54	0.15	0.77	0.08
Total					18.13	15.06	3.18		2.62		8.56		3.88		2.21		0.67

Table 2 : Effect of different doses of Gamma-rays EMS and their combination on frequency and spectrum of chlorophyll mutation in M_2 generation

VR-338(Kashi Gaurav)

		Chlorophyl mutants						Frequency and spectrum of chlorophyll mutants/1000 M_2 plant									
								Non viable mutants						Viable mutants			
	Dose	Number of M_2 plant	Number	Frequency /1000M2 plant	Total % of Chlorl. muts.	Total non viable muts.	Total viable muts.	Albino	%	Xantha	%	Dark Xantha	%	Viridis	%	Chlorina	%
Control		630.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10.00	1022.00	11.00	10.76	1.08	0.78	0.29	--	--	7.83	0.78	--	--	2.94	0.29	--	--
Gamma-rays	15.00	1017.00	13.00	12.78	1.28	1.18	0.10	3.93	0.39	5.90	0.59	1.97	0.20	0.98	0.10	--	--
(kR)	20.00	1086.00	14.00	12.89	1.29	0.64	0.65	--	--	1.84	0.18	4.60	0.46	2.76	0.28	3.68	0.37
	25.00	1155.00	15.00	12.99	1.30	1.30	--	4.33	0.43	6.93	0.69	1.73	0.17	--	--	--	--
	0.02	1133.00	10.00	8.83	0.88	0.44	0.44	0.88	0.09	0.88	0.09	2.65	0.26	3.53	0.35	0.88	0.09
EMS (M)	0.03	1245.00	11.00	8.84	0.88	0.72	0.16	--	--	4.02	0.40	3.21	0.32	1.61	0.16	--	--
	0.04	1028.00	12.00	11.67	1.17	0.58	0.58	0.97	0.10	4.86	0.49	--	--	5.84	0.58	--	--
	0.05	1189.00	14.00	11.77	1.18	1.09	0.08	5.89	0.59	--	--	5.05	0.50	--	--	0.84	0.08
	10.00	1061.00	6.00	5.66	0.57	0.47	0.09	0.94	0.09	1.89	0.19	1.89	0.19	0.94	0.09	--	--
Gamma-rays +	15.00	1012.00	10.00	9.88	0.99	0.99	--	--	--	7.91	0.79	1.98	0.20	--	--	--	--
0.03M EMS	20.00	1112.00	12.00	10.79	1.08	0.99	0.09	2.70	0.27	--	--	7.19	0.72	--	--	0.90	0.09
	25.00	1206.00	15.00	12.44	1.24	1.00	0.25	1.66	0.17	7.46	0.75	0.83	0.08	2.49	0.25	--	--
Total					12.93	10.19	2.73		2.13		4.95		3.11		2.11		0.63

mutagenically induced genetics alterations (Masken and Van der Veen,1968).

The chlorophyll mutation were increased with increasing dose of gamma-rays EMS and their combination in VR-338 variety of chilli but in variety of Kashi Anmol, mere EMS were enhanced the chlorophyll mutation with increasing concentration. The doses of gamma-rays enhanced the frequency of viable mutants than EMS and their combination in Kashi Anmol, while concentration of EMS enhanced the frequency of viable mutants than gamma-rays and their combination inVR-338. Chlorina and viridis (viable chlorophyll mutations) reported to be produced more at lower concentration of mutagen while, Albino, Xantha and Dark Xantha (non viable chlorophyll mutations) were recorded high frequently at the comparatively higher concentration of mutagens. Highest frequencies of chlorophyll mutations were found in Kashi anmol than VR-338 (Kashi Gaurav). Five different types of chlorophyll mutants produced in the agreement with the finding of several workers in the past. Ignacimuthu and Babu, 1988 reported Albino, Xantha Chlorina and viridis mutants in three species of vigna. Earlier studies have revealed as many as six types of chlorophyll mutations Gustaffson 1940. There was dose dependent increase in the spectrum and frequency of five types of chlorophyll mutations in M_2 similar result (Five types of chlorophyll mutations) were obtained black gram by Gaibriyal *et al.,* 2009. They reported that frequency and spectrum of chlorophyll mutations increased with doses, which is similar to the present study.

Conclusion :

The central matter in this mutation analysis concern the viable mutations, may of which, whether they are morphological or physiological in character, drastic or modifying, have potential value in plant breeding. High frequencies of chlorophyll mutation were found in gamma-rays doses than all the doses of EMS their combination in both variety of chilli. Although, genotypic differences in response to different doses of mutagens were observed. Broader spectrum of chlorophyll mutations was observed in EMS treatment of 0.02 and 0.03M in both the chilli varieties.

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