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Induced chlorophyll mutations in bell pepper (*Capsicum annuum* L. var. *grossum*)

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Abstract: An investigation entitled "Induced chlorophyll mutations in bell pepper (*Capsicum annuum* L. var. *grossum*)" was conducted during *kharif* (summer-rainy season) 2012 and 2013 at Experimental farm of the Department of Vegetable Science and Floriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, H.P., India. Healthy seeds of California Wonder of bell pepper were exposed to physical mutagen Gamma rays using ^{60}CO as a source of radiation at Mutation Breeding Centre, Department of Biotechnology, BARC Trombay, Mumbai and chemical mutagen EMS (Ethyl Methane Sulphonate) to obtain the spectrum and frequency of chlorophyll mutations in M_2 generation. The M_1 generation was produced from these mutagen treated seeds. Several unique and interesting chlorophyll and viable mutants were obtained in M_2 generation. In M_2 generation, gamma rays induced higher proportion of chlorophyll mutants then EMS. A progressive increase in mutation frequency of chlorophyll mutations was observed with increasing doses/concentrations. Four different types of chlorophyll mutants namely xantha, yellow xantha, chlorina and viridis were induced. Out of these mutants, chlorina and viridis were most frequent and were produced even in lower doses/concentrations while yellow xantha was least frequent and produced only in higher doses. The highest frequency of chlorophyll mutations (18.8 %) was reported in the 22 kR of gamma dose, while the lowest (0.80 %) frequency of chlorophyll mutations was found in the treatment of 1.0 % EMS. There was a dose dependent increase in the spectrum and frequency of chlorophyll mutations. These chlorophyll mutants induced by gamma radiation and EMS could be used in mutation breeding programme for inducing viable mutations for improvement of bell pepper varieties.

Keywords: Bell pepper, Chlorophyll mutants, Spectrum, Frequency, Gamma rays, Ethyl methane sulphonate

INTRODUCTION

The pepper species (*Capsicum annuum*L; 2n = 2x = 24) are important group of fruit vegetables and are ranked as second among important vegetable crops in the Solanaceae family after tomato (Bosland et al. 1996). Bell pepper (*Capsicum annuum* L. var. *grossum*Sendt.) also known as sweet pepper, green pepper, vegetable paprika or *Shimla mirch* is grown worldwide for its delicate taste, pleasant flavour and colour and is also the most leading crop under protected structures. Bell pepper fruits are generally blocky, square, thick fleshed, three to four lobed and non-pungent. Capsicum is native to Mexico with centre of diversity is in South America. Its fruits contain appreciable quantities of vitamin C (ascorbic acid), provitamin A (β-carotane) and other carotenoid pigments such as lycopene and zeaxanthin which are beneficial for prevention of cancer and cardiovascular human diseases. The pharmaceutical use of capsaicinoids is attributed to its antioxidant, anticancer, antiarthritic and analgesic properties.

In India, bell pepper was first introduced by the Britishers in the 19th century in Shimla hills and is commercially grown in Himachal Pradesh, Jammu and Kashmir, Uttarakhand, Arunachal Pradesh and Darjeeling district of West Bengal during summer-rainy months and as an autumn-winter crop in Maharashtra, Karnataka, Tamil Nadu and Bihar. In Himachal Pradesh, bell pepper holds a very coveted position as a leading off-season vegetable by generating cash revenues to the farmers by selling the produce in the neighbouring states and metropolitan cities. It is extensively grown as cash crop (June-October) in zone I, zone II and zone III in open environment.

Although breeders have made tremendous efforts for its genetic improvement, low yielding varieties are still being cultivated by the growers, resulting in the reduction in yield potential, quality and nutritive value of immature edible fruits of bell pepper.Many species of the Capsicum genus, are very poor from genetic improvement point of view (Rodrigues *et al*., 2012). Furthermore, productivity is low when compared to other countries. Hence, there is an urgent need to produce and identify new varieties combining high level of disease resistance, besides increased yield and coloured fruits. Chlorophyll mutations are considered as the most dependable indices for evaluating the efficiency of different mutagens in inducing the genetic variability for crop improvement and are also used as genetic markers in basic and applied research. The occurrence of chlorophyll mutations after treatments with physical and chemical mutagens have been reported in several crops Nascimento *et al.* (2015) worked on mutation breeding in Capsicum and reported occurrence of chlorophyll mutations in M_2 generation after treatment with mutagens gamma rays and EMS. Chlorophyll mutations offer one of the most reliable indices for the assessment of genetic effects of mutagenic treatments. Genotypic differences in response to induction of chlorophyll mutation can be observed in M_2 generations.

But with the help of mutagenesis which is one of the most critical steps for genetic studies as well as selective breeding desirable characters can be obtained. Various mutagenic agents are used to induce favorable mutations at high frequency that include ionizing radiations 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. These chemo and physico mutagens induce a broad variation of morphological and yield structure parameters in comparison to normal plants. Gene mutations influencing the green coloration of photo-synthetically active parts are among the most common induced alterations arising in higher plants. The chlorophyll mutation frequency is an indicator to predict the frequency of factor mutations and thus an index for evaluation of genetic effects of mutagens.Studies on effect of mutation in different crops e.g. Sridevi and Mullainathan (2011) in chilli; Kumar *et al*. (2012) in paprika and Vadluri *et al*. (2016) in capsicum also reported chlorophyll mutation frequency is an index of genetic effects of mutagens..Thus chlorophyll mutations offer one of the most reliable indices for the assessment of genetic effects of mutagenic treatments for evaluating the efficiency of different mutagens in inducing the genetic variability for crop improvement and are also used as genetic markers in basic and applied research. The occurrence of chlorophyll mutations after treatments with physical and chemical mutagens have been reported in several crops (Patil and Rane, (2015) in clusterbean; Ambli *et al*., (2016) in pearl millet and Bind *et al*., (2016) in cowpea).). Genotypic differences in response to induction of chlorophyll mutations can be observed as frequency of induced chlorophyll mutations in M_2 generation and the probability of occurrence of much category of mutation is obvious in all mutagen treatments. But most of the characterizations of different chlorophyll types in various crops occur only through morphology basis. Therefore, the present study was undertaken to gather information on the response of bell pepper to doses of irradiation and chemical mutagens in the form of chlorophyll mutations and characterized the chlorophyll types through biochemical chlorophyll quantification (most reliable method) and physical appearance basis.

MATERIALS AND METHODS

Healthy dry and uniform seeds of one commercial genotype i.e. California Wonder of bell pepper (*Capsicum annuum*L. *var. grossum*) were induced mutagenized by

Table 1. Spectrum and frequency of chlorophyll mutants in seedling stage in M_2 generation (Both gamma irradiated and EMS treated).

Treatments	Number of M_2 plants	Chlorophyll mutants		Spectrum/Frequency of chlorophyll mutants			
		Number	Frequency (%)	Xantha	Yellow-Xantha	Chlorina	Viridis
EMS							
1.0 %	2858	22	0.80	-	-	0.4	0.4
1.25%	2306	26	1.13	-	-	0.6	0.5
1.5%	1512	35	2.3	0.3	0.1	1.1	0.9
1.75%	495	32	6.5	0.4	-	3.6	2.7
2.0%	330	51	15.5	1.2	-	8.2	6.1
GAMMA IRRADIATION							
13kR	1743	40	2.9	-	-	1.0	0.6
16kR	675	37	5.5	0.4	-	2.8	2.2
19kR	407	72	17.7	1.7	0.7	9.1	6.1
22kR	173	32	18.8	2.9	-	10.4	5.2

Table 2. Pigment content of chlorophyll mutants.

Types of chlorophyll mutants	Chlorophyll (µg per g of fresh plant)
Xantha - (dark yellow, lethal)	>20-40
Yellow xantha- (yellow to yellowish white, lethal)	>40-100
Viridis- (uniform green colour with white on tips, viable)	>100-500
Chlorina- (uniform light yellow green colour of leaves, viable)	>100-500
Normal type- (along with control)	>500-1000

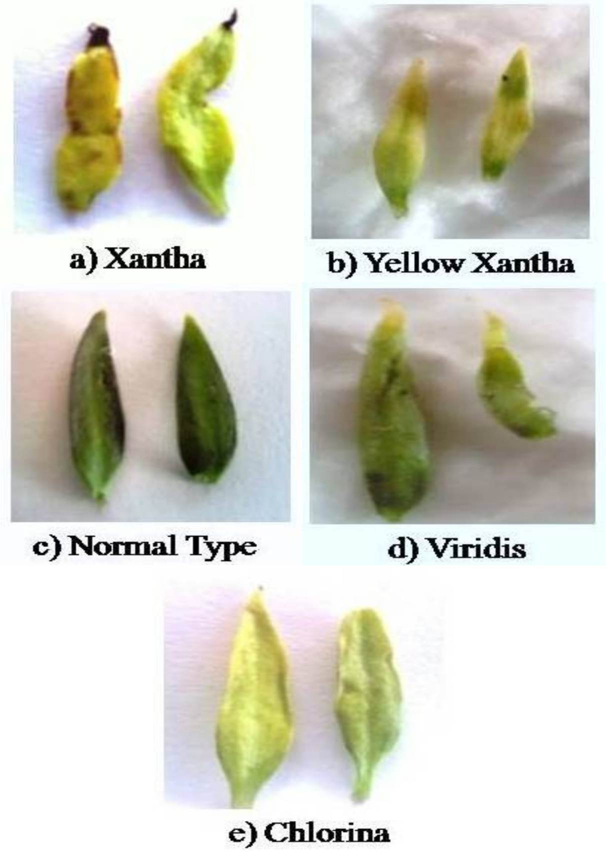

Fig. 1 (a, b, c, d and e). *Different types of chlorophyll mutants in seedling stage.*

physical (Gamma rays) and chemical mutagen (EMS).

Gamma rays treatment: Selected seeds of bell pepper were irradiated from a ^{60}CO source at Bombay Atomic Research Centre (BARC), Trombay, Mumbai with a dose of 0.5, 1.0, 3.0, 5.0, 8.0, 11, 13, 16, 19 and 22kR.

EMS treatment: For chemical mutagen treatment, seeds were pre-soaked for 12 hours in distilled water, blotted dry, and treated with freshly prepared aqueous solution of ethyl methane sulphonate (EMS) at different concentrations (0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 2.0 and 3.0 %) for 5.30 hours with shaking at intermittent intervals as described by Alcantra *et al.* (1996).After the treatment, the seeds were washed thoroughly with distilled water for eight to ten times to leach out residual chemicals.

Both physical gamma rays irradiated and chemically treated (EMS) seeds along with control (untreated seeds) were sown in the Experimental farm of the Department of Vegetable Science and Floriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur (32°6' N, 76°3' E), H. P., India to find out LD_{50} and to raise M_1 generation in 2012. On the basis of LD_{50} in both mutagens (physical and chemical) following doses were selected for further mutation screening.

Gamma irradiated (physical mutagen): 13kR, 16kR, 19 kR and 22kR

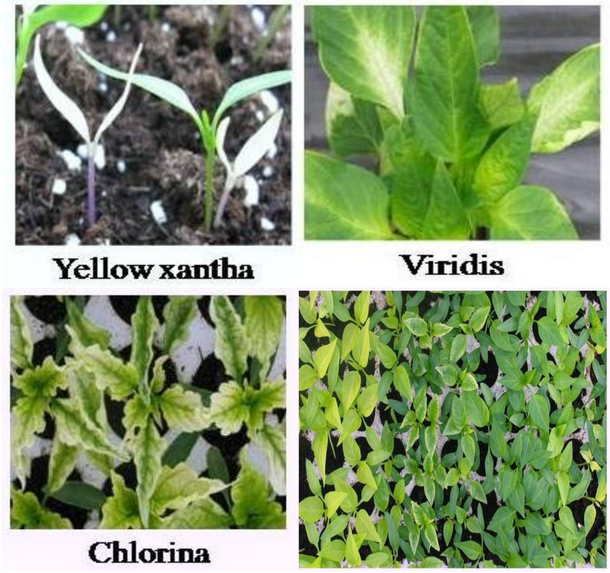

Fig. 2. *Chlorophyll mutants i.e. Xantha, Viridis, Chlorina, normal types and yellow xantha in plugtray.*

EMS treatment (chemical treatment): 1.0 %, 1.25 %, 1.5 %, 1.75 % and 2.0 %

All surviving M_1 plants were selfed and harvested to get M_2 seeds. M_2 generations were raised in 2013 to screen chlorophyll mutations on morphology as well as biochemical basis and chlorophyll quantification was also done according to Hiscox and Israelstam, 1979. Lethal chlorophyll mutations were scored within 10 to 25 days of sowing whereas viable chlorophyll mutations were scored throughout the life cycle of plants. The spectrum of chlorophyll mutations was studied and the mutants were classified as per the scheme of Gustafson (1940) with modification; Albino, Yellow Xantha, Dark xantha, Chlorina and Viridis.

RESULTS AND DISCUSSION

Mutation frequency was estimated on the basis of phenotype, using screens for seedling lethality (survival rate), embryonic lethality (seed set), chlorophyll deficiency or single-copy gene phenotypes as a measure. Frequencies and types of the chlorophyll mutations observed in present study were albino, xantha, yellow xantha, chlorina, viridis and lutescens etc. Generally, gamma rays induced higher proportion of chlorophyll mutants than EMS. In *S.villosum*, the earliest observable mutants were chlorophyll deficiency mutants (Gustafson, 1940) whose frequencies varied with the mutagen dose and male sterile mutant frequency was later observed to follow a similar distribution trend (Ojiewo *et al.,* 2006).

Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in various crops. In the present experiment, data were recorded on the frequency of chlorophyll mutations in M_2 plants (Table 1). Chlorophyll mutations were found in almost all the mutagenic treatments. High frequency of chlorophyll mutations were found in the higher dose of gamma rays. The highest frequency of chlorophyll mutations (18.8 %) was reported in the 22 kR of gamma dose, while the lowest (0.80 %) frequency of chlorophyll mutations was found with the treatment of 1.0 % EMS. Gamma ray was found to be more effective for inducing chlorophyll mutations in comparison to EMS. Their spectrum and frequency of chlorophyll mutants (classified as the scheme of Gustafson, 1940 with modification) at seedling stage in different doses of gamma and treatments of EMS are shown in Table 1. The spectrum of chlorophyll mutations in segregating M_2 generation indicates presence of broad chlorophyll mutant spectrum comprising 4 types (maximum) was induced by 19 kR followed by 1.5 % EMS treatment which also include four types of chlorophyll mutants Fig 2. (Fig Chlorina mutant recorded highest frequency (10.4) in 22 kR treatment followed by viridis mutants with highest frequency (6.1) in 2.0 % EMS and xantha (2.9) in 22 kR. Chlorina and viridis mutants observed in all the doses of both mutagens. In a study conducted by Gaur *et al*. (2013) in capsicum, Xantha type of chlorophyll mutation recorded the highest frequency followed by Dark Xantha, Albina, Viridis, and Chlorina.

Depiction of chlorophyll mutants on morphology basis is mystifying method (Fig 1), but it can be done through chlorophyll quantification (biochemically) which is consistent one. On morphological basis, it is hard to distinguish the xantha and yellow xantha chlorophyll mutants in some cases, but these mutants are easy to characterize on the basis of total chlorophyll contents (Table 2). The various mutation types are classified according to the following pigment characterization. Although viridis and chlorina have same chlorophyll content they are different in leaves prototype (Figs. 1 d,e).From Table 1, it is evident that frequency of chlorophyll mutations increases proportionally with mutagen dose up to saturation point (Waghmareand Mehra, 2001; Firdose *et al*., 2011). The spectrum/frequency of chlorophyll mutants was independent of mutagen dose. This was not similar to results obtained in other crops, Ignacimuthu and Babu, 1988 observed that there was increase in spectrum and frequency of chlorophyll mutants with increase in dosage of mutagen. Manju *et al*. (1983) observed that chlorophyll mutation frequency in M_2 seedling showed dose dependence in horse gram. Similarly, Srideviand Mullainathan (2011) in chilli, Patil and Rane (2015) in clusterbean and Valduri *et al*., (2016) in capsicum also showed similar results which is in agreement with the present study. Viable chlorophyll mutations, i.e., chlorina and viridis were produced more at lower doses/concentrations of mutagen whereas lethal mutants (xantha and yellow xantha) were observed more frequently at relatively higher doses/concentrations of the mutagens.

Even though the chlorophyll mutations do not have any economic value, due to their lethal nature, such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability. Mutagenesis inducing chlorophyll mutations in bell pepper, also creates a scope for producing fruits of different colors (green, yellow, orange, red and violet) acceptable to consumers. However, there is a need to segregate the lethal chlorophyll mutants (xantha, yellow xantha) from the non-lethal ones (viridis and chlorina). The later two types of chlorophyll mutants increase the probability for getting coloured fruits. Mutation spectra can be altered in various ways by the application of different mutagens. This is valid not only with regard to the types of chlorophyll mutations but also with regard to the rates of translocation sterility and viable mutations.

Conclusion

In the present investigation, the frequency of chlorophyll mutations was increased according to increasing dose of gamma rays and EMS. It was higher at higher doses (22 kR) and (2 %) and lower at lower doses of (13 kR) and (1 %) of gamma rays and EMS, respectively. Highest chlorophyll mutant spectrum was noticed in 22 kR gamma rays treatment. Four types of chlorophyll mutants were recorded viz., Xantha, Yellow Xantha, Chlorina and Viridis. Out of that Xantha and Yellow Xantha were lethal and Chlorina and Viridis were viable mutants. Chlorina was most frequent whereas Yellow Xantha was less frequent. Maximum spectrum of Chlorina chlorophyll mutations i.e. 8.2 and 10.4 was observed in the higher doses of EMS and gamma rays, respectively. The spectrum of chlorophyll mutations was dose dependent and they have been appeared in irregular pattern in bell pepper var. California Wonder. These chlorophyll mutants induced by gamma rays and EMS could be used in mutation breeding programmes for inducing viable mutations for improvement of bell pepper varieties.

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