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Research Note

Spectrum and frequency of mutations induced by gamma rays and EMS in okra [*Abelmoschus esculentus* (L.) Moench]

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Abstract
The seeds of okra variety P-8 were treated with different concentrations of gamma rays (65kR, 75kR and 85kR), EMS (1.2%, 1.4% and 1.6%) and their combinations to obtain the phenotypic response during M_2 generation. Different mutation frequencies and width of mutation spectra were induced under the action of different concentrations of the applied mutagens. A progressive increase in mutation frequency of chlorophyll mutations was observed with increasing doses. Four different types of chlorophyll mutants, such as albino, xantha, viridis and chlorina were induced with effect of mutagens on plant population. The spectrum and frequency of chlorina mutants were highest followed by xantha, viridis and albino in all the treatments. The sole treatments of mutagens were found to be more efficient than combinations in inducing chlorophyll mutations. The highest frequency of chlorophyll mutations (0.044%) was reported in the 75kR+1.2% EMS. Based on the chlorophyll mutation frequency, it was found that EMS was most effective followed by gamma rays and combination of treatments.

Key words
Okra, Gamma rays, EMS, Chlorophyll mutations.

Okra [*Abelmoschus esculentus* (L.) Moench] is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. Due to high nutritive value and long post harvest life, okra has captured a prominent position among export vegetables. It has a vast potential to earn foreign exchange and accounts for 60 percent export of fresh vegetables excluding potato, onion and garlic, the destinations being the Middle East, United Kingdom, Western Europe and United States of America. Fresh okra pod is an excellent source of vitamins A and C, and calcium (NARP, 1993). It also contains carbohydrates, potassium, magnesium and other vitamins at a significant level . There were also reports about anticancer (Vayssade *et al*., 2010), antimicrobial (De Carvalho *et al*., 2011), antiulcer (Atodariya *et al*., 2013), antihyperlipidemic (Sabitha *et al*., 2011) and hypoglycaemic activities (Tomoda *et al*., 1989, Sabitha *et al*., 2011) of okra.

Genetic diversity in okra is lost through fixation, genetic sweeps thereby increasing the dependency of breeders on smaller sets of superior genotypes that has created successive bottlenecks (Varshney, 2013).

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. Chlorophyll mutants are employed as markers for the evaluation of gene action of mutagenic factors in induced mutation studies (Gaul, 1964). The mutagenic effect is reflected in the segregation of chlorophyll mutants. The present investigation was attempted to understand the comparative response of physical mutagen gamma rays and chemical mutagens EMS and combination of them on okra, with a view to determine the genotypic differences in response to induction of chlorophyll mutations in M_2 generation.

The experiment was conducted at Department of Vegetable Science and Floriculture, CSK HPKV, Palampur, Himachal Pradesh, during *kharif* season 2014-2016. The experimental area comes under sub-temperate zone. A well adapted variety of okra P-8 had been used for this experiment. The plants of P-8 are 150 cm tall with purple pigmentation on stem, petiole and lower leaf surface having sparse hairs. Fruits of P-8 are medium long, thin, tender, green and five ridged. It had been released for general cultivation in Himachal Pradesh during 2010. Different mutagens like gamma rays and EMS were used to mutagenize the seeds. Six hundred healthy, well filled, mature and disease free dry seeds of P-8 variety of okra were irradiated with different levels of gamma rays *viz*., 35 kR, 40 kR, 45 kR, 50 kR, 55 kR, 60 kR, 65 kR, 70 kR, 75 kR, 80 kR and 85 kR of gamma rays in ^{60}Co gamma-cell at Gamma chamber, Bhabha Atomic Research Centre, Trombay, Mumbai, India.

Prior to chemical mutagenic treatments, well-filled, mature and disease free seeds of the P-8 variety were immersed in distilled water for 6

hours. The pre-soaking enhances the rate of uptake of the mutagen through increase in cell permeability and also initiates metabolism in seeds. Such pre-soaked seeds were later immersed in the mutagenic solution for 6 hours with intermittent shaking followed by 12 hours post treatment washing to wash out chemical residues. The different concentrations of EMS used for the chemical mutagenic treatments were 0.2%, 0.4%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.4% and 1.6%. Seeds soaked in distilled water for 6 hours served as control.

Treated seeds of each dose were placed in Petri plates over moistened germination paper under seed germinator with untreated seeds as control at 25 ± 1^0 C under laboratory conditions in three replications to work out LD_{50} value by recording germination percentage and seedling length.

On the basis of LD_{50} values (75kR for gamma rays and 1.2% for EMS), the preceding and succeeding doses of gamma rays (65kR, 85kR) and EMS (1.4%, 1.6%) were selected for further experiments. The treatment was given individually as well as in combination with each other (Table1). Harvested seeds were sown as single plant to progeny in M_2 generation along with control in rows of 2.25m length maintaining plant to plant and row to row spacing of 15 cm and 45 cm, respectively in augmented design. The recommended agronomic practices were followed to raise a good crop. The M_2 population was screened for frequency and spectrum of chlorophyll mutations. Lethal chlorophyll mutations were scored within 10 to 25 days after sowing, whereas viable chlorophyll mutations were scored throughout the cropping season. The spectrum of chlorophyll mutations was studied and the mutants were classified as per the scheme of Gustafsson (1940) and Blixt (1969).

The frequency of chlorophyll mutations was calculated by the following formula: Mutation frequency = Number of mutant plants/ Total number of M_2 plants × 100

Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments. Chlorophyll biosynthesis has been suggested to be controlled by several genes located adjacent to centromere and proximal part of chromosomes (Goud, 1967). Mutations in these chlorophyll genes are expressed in the M_2 and coming generations in the form of various types of mutants. To estimate the chlorophyll mutations, periodical scoring of plants is essential right from the seedling stage (Stumman *et al*., 1980).

The spectrum of chlorophyll mutations were classified as per the scheme of Gustafson (1947).

In the present investigation, after recording the data on seedling length and germination percentage, LD_{50} values for gamma rays and EMS was observed as 65kR and 1.4%, respectively. In M_2 generation, four different types of chlorophyll mutants were recorded for the different doses/ concentrations of gamma rays, EMS and their combinations *i.e.* albino, xantha, viridis and chlorina. Albino mutants were completely devoid of chlorophyll and could survive only a few days. The seedlings emerged white on germination and relatively smaller than the normal looking seedlings of same age, Xantha had pale yellow coloured seedlings, these mutants could not survive more than a few days due to block in chlorophyll synthesis. They all showed normal growth in the beginning, but withering after 8-10 days and ultimately died within two week (Blixt, 1961). Both these mutants were classified under lethal mutants.

Several other types of chlorophyll deficient mutants such as chlorina and viridis (viable mutants) survived till maturity. Viridis mutants were light greenish in colour and remained so throughout the life cycle. Chlorina mutant seedlings were pale yellowish to yellow in colour. Some of these mutants were lethal or semi-lethal, few plants acquire greenish tinge, later fade in colour. The plants were semi dwarf to normal in height.

Poudel *et al*., 2016 observed different types of chlorophyll mutants *viz.,* Albino, Xantha, Chlorina and Viridis and found Viridis and Chlorina type of chlorophyll mutations were most frequent and least common, respectively. Significant variation in the spectrum and frequency of different chlorophyll mutations was recorded among the different treatments.

The chlorophyll mutation frequency was estimated as percentage of plants that segregated for chlorophyll deficiency on the basis of M_2 seedlings and presented in Table 2. The highest frequency of chlorophyll mutations (9.74%) was recorded in the 65kR+1.6% EMS treatment while lowest (0.004%) was found in 1.6% EMS treatment.

Out of 15 different treatments, all four types of chlorophyll mutations were induced by 1.4% EMS treatment followed by 75kR and 75kR+1.2% EMS treatments which induced 3 types of chlorophyll mutants. Also chlorina mutants were dominant followed by xantha, viridis and albino. Highest spectrum of mutants was shown by 1.4% EMS (9) followed by 75kR (5) and both combinations of 65kR+1.6% EMS and 75kR+1.2% EMS (4). Highest frequency was found in 75kR+1.2% EMS (0.044%) followed by 65kR+1.6% EMS (0.028%) and 1.4% EMS (0.024%).

The chlorophyll mutation acts not only as a scale for evaluating effectiveness and efficiency of mutagens, but also as indicators to predict the size of vital factor mutations These studies aimed at understanding the process of mutation, testing the efficacy of various mutagens, identifying optimum dose and best method of treatment, also isolates the mutants of basic and applied value which elucidates the biological effects of mutagens. Out of albina, chlorina, viresence, viridis and xantha; xantha type of chlorophyll mutations was predominant in both mutagenic treatments (Kausar *et al*., 2013). The albino seedling itself has no practical value; however, such seedlings may be used as genetic markers for estimation of natural selfing. The phenomenon of albinism is rarely exhibited by plants with characteristic deficiency of chlorophyll and subsequent whitish-yellow colour.

In the present investigation no dose dependent increase in the frequency and spectrum of chlorophyll mutations was observed. Chlorophyll mutations both lethal and viable have been shown in Fig 1 and Fig 2, respectively. For both EMS and gamma irradiation higher frequency of chlorophyll mutation with moderate doses of mutagens was observed. It seems that the strong mutagens reach their saturation point even at moderate doses in the highly mutable genotypes and further increase in dose does not add to the mutation frequency. With increase in dose beyond a point, the strong mutagens become more toxic than the higher doses of relatively weaker mutagens. That's why in combination treatments with higher doses of both mutagens, no chlorophyll mutants were observed. The present findings are in accordance with Shah *et al*., 2006; the viridis type chlorophyll mutations were most frequent and albina were least common. Among the mutagens, chemical mutagen (EMS) was most effective and potent in inducing the chlorophyll mutations than physical mutagen gamma irradiation.

Induced mutations have been recognized as an important tool for crop improvement, and have sufficient scope in okra. Delayed in the development of chloroplast leading to chlorophyll deficient mutants. Mutations in these chlorophyll genes are expressed in the M_2 and coming after generations in the form of various types of mutants. It is therefore concluded in pigeonpea, that although 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 and number of economically useful mutants in the segregating generations (Sangle and Kothekar, 2013). Reddy and Chaudhary (2015) observed that gamma irradiation treatments seemed to be superior in producing greater frequencies over all doses in okra germplasm. The occurrence of chlorophyll mutations after treatments with physical and chemical mutagens have also been reported in okra by different workers, *viz*., Jambhale and Nerkar (1982), Singh *et al*. (2000), Sasi *et al*. (2005), Kumar and Mishra (2006), Mishra *et al*. (2007) and Warghat *et al*. (2011). Khursheed and Khan (2016) in *Vicia faba* also reported that the frequency of chlorophyll mutations increased with increasing concentrations of both single and combined treatments. Combined treatments produced more chlorophyll mutations followed by individual concentrations/doses of EMS and gamma rays. Reason for higher frequency of chlorophyll mutants in combination treatments may be due to higher induction of mutations in genes controlling chlorophyll biosynthesis. Further isolation and characterization of chlorophyll genome of mutant may be helpful in understanding the nature of mutation at molecular level.

The present investigation revealed how chlorophyll genes respond to mutagens gamma rays and EMS. Chlorophyll genes are reflected in the M_2 in the form of different types of chlorophyll mutants which can be useful as marker in physiological and biochemical investigations. Among the different treatments, 1.4% EMS showed maximum spectrum and maximum frequency of chlorophyll mutants was found in 75kR+1.2% EMS. Chlorina type of chlorophyll mutations was most frequent and Albino type was least common. Based on the chlorophyll mutation frequency, EMS was most effective followed by gamma rays and combination of treatments. It is further suggested that as all the gamma radiations and EMS doses induced reasonable chlorophyll mutations, hence all these treatments could be used in mutation breeding programs for inducing viable mutations.

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Table 1. Mutagen treatment based on lethal dose

S.No.	Dose	Mutagen	Remarks
1.	65kR	Gamma rays	Below LD_{50}
2.	75kR	Gamma rays	LD_{50}
3.	85kR	Gamma rays	Above LD_{50}
4.	1.2%	EMS	Below LD_{50}
5.	1.4%	EMS	LD_{50}
6.	1.6%	EMS	Above LD_{50}
7.	65 kR+ 1.2%	Gamma rays+ EMS	Combination of below LD_{50} gamma rays and below LD_{50} EMS
8.	65kR+1.4%	Gamma rays+ EMS	Combination of below LD_{50} gamma rays and LD_{50} EMS
9.	65 kR+1.6%	Gamma rays+ EMS	Combination of below LD_{50} gamma rays and above LD_{50} EMS
10.	75 kR +1.2%	Gamma rays+ EMS	Combination of LD_{50} gamma rays and below LD_{50} EMS
11.	75 kR +1.4%	Gamma rays+ EMS	Combination of LD_{50} gamma rays and LD_{50} EMS
12.	75 kR +1.6%	Gamma rays+ EMS	Combination of LD_{50} gamma rays and above LD_{50} EMS
13.	85 kR +1.2%	Gamma rays+ EMS	Combination of above LD_{50} gamma rays and below LD_{50} EMS
14.	85 kR+1.4%	Gamma rays+ EMS	Combination of above LD_{50} gamma rays and LD_{50} EMS
15.	85 kR+1.6%	Gamma rays+ EMS	Combination of above LD_{50} gamma rays and above LD_{50} EMS

Table 2. Spectrum and frequency of chlorophyll mutants in M_2 generation of okra

Treatments	Total number of M_2 plants	Albino	Xantha	Viridis	Chlorina	Total
65 kR	200	1(0.005)	-	-	1(0.005)	2(0.010)
75 kR	480	-	1(0.002)	2(0.004)	2(0.004)	5(0.010)
85 kR	250	-	-	-	1(0.004)	1(0.004)
1.2% EMS	650	-	1(0.002)	-	-	1(0.002)
1.4% EMS	600	2(0.003)	2(0.003)	2(0.003)	3(0.015)	9(0.024)
1.6% EMS	606	-	1(0.002)	-	1(0.002)	2(0.004)
65kR+1.2% EMS	108	1(0.009)	-	-	-	1(0.009)
65kR+1.4% EMS	132	-	2(0.015)	-	-	2(0.015)
65kR+1.6% EMS	140	1(0.007)	-	1(0.007)	2(0.014)	4(0.028)
75kR+1.2% EMS	120	-	1(0.011)	2(0.022)	1(0.011)	4(0.044)
75kR+1.4% EMS	162	-	-	-	3(0.019)	3(0.019)
75kR+1.6% EMS	15	-	-	-	-	-
85kR+1.2% EMS	35	-	-	-	-	-
85kR+1.4% EMS	90	-	-	-	-	-
85kR+1.6% EMS	15	-	-	-	-	-

Note: The values outside parentheses refer to the spectrum while values in parentheses refer to the frequency.

65kR

1.4% EMS

a) Albino

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1.6% EMS **65kR+ 1.4% EMS**

b) Xantha

Fig. 1(a,b). Lethal chlorophyll mutants (albino and xantha) in M_2 generation of okra

75kR **75kR+ 1.2% EMS**

c) Viridis

85kR **75kR+ 1.4% EMS**

d) Chlorina

Fig. 2. (c,d): Viable chlorophyll mutants (viridis and chlorina) in M_2 generation of okra