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Mutagenic Effects of Gamma Rays and EMS on Frequency and Spectrum of Chlorophyll Mutations in Pea (*Pisum sativum* L.)

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Abstract

The mutagenic effect of gamma rays (5kR, 7kR and 10kR) and ethyl methanesulphonate (0.05%, 0.10% and 0.15%) on frequency and spectrum of chlorophyll mutations in two varieties of pea, namely, DDR-53 and DMR-55 have been observed. Conclusively the various doses or concentrations of mutagenic treatments have independent response towards frequency and spectrum of chlorophyll mutations.

1. Introduction

Pea (*Pisum sativum* L.) is a cool season annual crop produced worldwide for human consumption and animal feed. The garden pea is widely cultivated one and grouped as *Pisum sativum* L. subspecies *hortense* and the field pea as *Pisum sativum* L. subspecies *arvense*. Edible podded peas are found in both these subspecies. Several investigators hold *Pisum sativum* and *Pisum arvense* to be one species. Govorov (1928) suggested the inclusion of all cultivated forms of peas in one species *Pisum sativum* L. and subdivided it into two subspecies viz. *sativum* L. and *arvense* L. Menjkova (1954) observed that *Pisum sativum* and *Pisum arvense* crossed readily and gave fertile progeny and normal segregates. He concluded that the two kinds pertain to the same species. Both of these have the same chromosome number 2n=14. Mutation breeding is relatively a quicker method for improvement of crops. It has been observed that induced mutations can increase yield as well as other quantitative traits in plants. The choice of mutagen holds great importance in changing the frequency and spectrum of chlorophyll mutations in a predictable manner. The physical and chemical mutagens cause three types of effects i.e. physical damage, gene mutation and chromosomal aberrations (Swaminathan,1965). Induction of chlorophyll mutations in general is considered as a measure to assess the effectiveness of various mutagens. In the present study, the effect of gamma rays and ethyl methanesulphonate was studied on the frequency and spectrum of chlorophyll mutations in M_2 generation of pea.

2. Material and Methods

Healthy, uniform and dry seeds of two varieties of pea (*Pisum sativum* L.), namely, DDR-53 and DMR-55 were exposed to ^{60}Co gamma rays each 5kR, 7kR and 10kR doses at Department of Biophysics, Government Institute of Science, Aurangabad (M.S.). Separate seed lots of these varieties were presoaked in distilled water for 6 hrs. The soaked seeds were treated with 0.05%, 0.10% and0.15% EMS for 6 hrs. (P^H 7.0). The treated seeds were washed in running tap water. The 100 seeds from each treatments along with control were placed in petridishes in laboratory for taking observations on seed germination and seedling height. Rest of the treated seeds were sown in a randomized block design (RBD) with three replications each consisting of 100 seeds along with control for raising M_1 generation. The seeds were sown at the rate of 100 seeds per plot at a spacing of 15 X 40 cm within and between rows in the field during rabbi season of 2004. The M_2 generation was raised from individual M_1 plants in the field in next season 2005. The M2 populations were screened for both frequency and spectrum of different types of chlorophyll mutations at various developmental stages, particularly from flowering to maturity period. The classification and characterization of various chlorophyll mutants was done according to Gustafsson (1940) and Bilxt (1961) and the spectrum was recorded as *xantha, chlorina, viridis* and *albina*. The *xantha* mutants displayed a bright yellow to deep golden yellow colour. *Chlorina* mutants were yellowish green in colour, the *viridis* mutants displayed light green colour and *albina* shows white colour. The chlorophyll mutants like *xantha, chlorina* and *viridis* survived till maturity. But *albina* did not survive for many days and it proved to be lethal.

3. Results and Discussion

Table-1. Effect of mutagens on the frequency of chlorophyll mutants in M_2 generation of pea (*Pisum sativum* L.) variety-DDR-53 and DMR-55

Mutagens	Concentration / Dose	No. of plants observed	Total No. of chlorophyll mutants	Frequency of chlorophyll Mutants (MF)
Control (DDR-53)		280	-	-
	0.05	248	8	3.22
EMS (%)	0.10	260	10	3.84
	0.15	252	14	5.55
	5 kR	268	7	2.61
Gamma rays	7 kR	275	9	3.27
	10 kR	255	16	6.27
Control (DMR-55)		285	-	-
	0.05	260	9	3.46
EMS (%)	0.10	272	13	4.77
	0.15	266	15	5.63
	5 kR	270	11	4.07
Gamma rays	7 kR	282	14	4.96
	10 kR	266	16	6.01

It is evident from the data (Table-1) that higher doses or concentrations of mutagens were more effective in inducing greater frequency of chlorophyll mutations in both the varieties. The highest frequency of chlorophyll mutations (6.27%) was induced at 10 kR dose of gamma ray treatment, while the lowest frequency of chlorophyll mutations (2.61%) was induced at 5kR dose of gamma ray treatment in variety DDR-53. As regards variety DMR-55, the frequency of chlorophyll mutations varied from (3.46% to 6.01%) after both the mutagenic treatments. The highest frequency of chlorophyll mutations (6.01%) was recorded at 10kR dose of gamma ray treatment, while the lowest frequency (3.46%) was induced at 0.05% EMS concentration. Marked varietal differences were present in terms of induction of chlorophyll mutations at different doses or concentrations of the mutagens. Gamma rays proved to be the most effective mutagenic agent for inducing chlorophyll mutations in both the varieties than EMS concentrations. Between the varieties under reference, DMR-55 exhibited a higher response in terms of induction of mutations than variety DDR-53.

Table- 2. Effect of mutagens on relative spectrum of chlorophyll mutants in M_2 generation of pea (*Pisum sativum* L.) variety-DDR-53 and DMR-55

Variety DDR-53	Concentration / Dose	Relative spectrum of chlorophyll mutants			
Mutagens		*Xantha*	*chlorina*	*viridis*	*Albina*
	0.05	50.00	37.50	12.50	-
EMS (%)	0.10	40.00	40.00	20.00	-
	0.15	42.85	35.71	7.14	7.14
	5 kR	28.50	57.14	14.28	-
Gamma rays	7 kR	44.44	33.33	11.11	11.11
	10 kR	43.75	31.25	12.50	12.50

Variety DMR-55		*xantha*	*chlorina*	*viridis*	*Albina*
	0.05	44.44	44.44	11.11	-
EMS (%)	0.10	30.76	46.15	15.38	7.69
	0.15	26.66	46.66	6.66	6.66
	5 kR	36.36	45.45	9.09	9.09
Gamma rays	7 kR	35.71	42.85	14.28	7.14
	10 kR	31.25	43.75	12.50	12.50

The spectrum of chlorophyll mutations observed in segregating M_2 generation of both varieties were analyzed and their respective frequencies are given in table-2.

In general the frequency of relative spectrum of chlorophyll mutation was higher in variety DDR-53 than variety DMR-55. The frequency of different types of chlorophyll mutations revealed that the occurance of *xantha* mutatnts revealed maximum percentage (50.00 and 44.44) at 0.05% EMS concentration in both varieties of pea. It was found that the *chlorina* type of mutant could get induced maximally at 5 kR dose of gamma ray treatment(57.14) in variety DDR-53, while it was (46.66%) at 0.15% EMS concentration in variety DMR-55. The *viridis* type of chlorophyll mutant revealed its highest relative percentage (20.00) at 0.10% EMS concentration in variety DDR-53. The *albina* type of chlorophyll mutant revealed its highest relative percentage (12.50) at 10 kR dose of gamma rays treatment in both the varieties of pea.

The frequency of induced chlorophyll mutations in M_2 generation has been considered as a reliable index for noting the potency of mutagens due to greater accuracy in their scoring (Gustafsson, 1940; and Mackey, 1951). The chlorophyll mutation serve not only as a measure for evaluating effectiveness and efficiency of mutagens, but also as indicators to predict the size of vital factor mutations.

The higher dose of gamma ray treatment promoted larger chlorophyll mutation spectrum in grass pea (Das and Kundagrami, 2000). The occurance of chlorophyll mutations after mutagenic treatment has been reported in pigeon pea (Venkateswarlu, et. al., 1978), lentil (Dixit and Dubey, 1986) and mungbean (Singh and Yadav, 1991). Chlorophyll development seems to be controlled by many genes located on several chromosomes (Goud, 1967) which could be adjacent to centromere and proximal segments of chromosomes (Swaminathan, 1964 and 1965). Mutations in these chlorophyll genes may induce chlorophyll mutations. The high incidence of chlorophyll mutation induced by EMS may be due to its specificity to affect certain region of the chromosomes (Natrajan and Upadhaya, 1964). Alkylating agents induce less drastic chlorophyll mutants such as *albina* in higher proportion than those induced by radiations, probably because of their apparently less drastic effect on chromosomes (Nilan, 1972). The origin of chlorophyll deficiencies is mainly due to mutations in genes, which are responsible for synthesis of photosynthetic pigments. The chlorophyll mutants are usually lethal but semilethal and viable mutants are also known (Kothekar, et. al., 1994).

In the present study, a wide spectrum of chlorophyll mutants has been recorded. The different chlorophyll mutants were of the types such as *xantha, chlorina, viridis* and *albina*. The *xantha* was found to be most abundant type in variety DDR-53, while the *chlorina* was found to be most abundant type in variety DMR-55 in both the mutagenic treatments. These findings are in agreement with earlier reports of Singh, et. al., (1999) and Singh, et. al., (2000).

The frequency of mutants was found independent of the doses of gamma rays (Bekendam, 1961;Reddi and Rao, 1988). Among the mutants scored, *xantha* was the most frequent (Singh, et. al., 1998). Paul and Singh (2002) in lentil noticed differential response of genotypes to irradiation doses in respect of frequency of chlorophyll mutations.

The chlorophyll mutation frequency in the present study increased with increasing concentration or dose of mutagens. Similar results were observed by Sudha Rani (1990), Gautam, *et. al.*, (1992) and Rayyan (1995) in different plants.

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