

Original Research Article

Induced chlorophyll mutation in grasspea (*Lathyrus Sativus* Linn.)

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A B S T R A C T

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Variety Pusa -24 were treated with Gamma rays, EMS and Combination treatment of Gamma rays with EMS to obtain the spectrum and frequency of chlorophyll mutations in M2 generation in the present investigation. The individual treatment of EMS was found to be more efficient than gamma rays to induce chlorophyll mutants. A progressive increase in mutation frequency of chlorophyll mutations was observed with increasing doses. Three different types of chlorophyll mutants, such as, Albina, Xantha and Viridis were induced with effect of mutagens. The highest frequency of chlorophyll mutations were observed in the combination of EMS and Gamma Rays . There was a dose dependent increase in the spectrum and frequency of chlorophyll mutations whether mutagens were employed singly or in combination.

Introduction

Grass pea (*Lathyrus sativus* L.) belonging to the family Leguminosae is an annual, herbaceous popular food crop in many Asian and African countries where it is grown for human consumption and as animal feedstock. Its domestication began in Balkan Peninsula in the early Neolithic period and its cultivation has now spread from The Mediterranean basin to the Europe, Australia and South America (Smartt, 1984; Kislev, 1989; McCutchan, 2003). It is the only cultivated grain legume within the genus *Lathyrus* and showed tolerance to drought as well as water logging, resistance to insects and

pests, adaptability to nearly all types of soils and adverse climatic conditions and high seed protein content (Campbell, 1997; Biswas, 2007). Mutation breeding is accomplished by chemical or physical treatments followed by selection for heritable changes of specific genotypes, and this method has been used successfully in the genetic improvement of crop plants (Mick *et al.*, 1985). Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (Subuthi *et al.*, 1991). Gene mutations influencing the green coloration of photo synthetically active

parts are among the most common spontaneous or 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 (Gustafsson, 1951; D'Amato *et al.*, 1962; Gichner and Veleminsky, 1965). Nuclear gene mutations or extra chromosomal mutations might result in chlorophyll deficient mutations (Levine, 1972; Walles, 1973 and Wildman, 1973). 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 (Swaminathan *et al.*, 1962; Prasad and Das, 1980; Sharma and Sharma, 1981; Reddy and Gupta, 1989; Kharkwal, 1998; Mitra and Bhowmik, 1999 and Solanki, 2005). In the present investigation was attempted to made to understand the comparative response of physical mutagen gamma rays and chemical mutagens EMS an combination of them on Grasspea , with a view to determine the mutagen and treatment causing genotypic differences in response to induction of chlorophyll mutations can be observed as frequency of induced chlorophyll mutations in M₂ generation.

Materials and Methods

The dry seeds of Grasspea (*Lathyrus sativus linn.*) variety PUSA_24 were treated with Gamma rays, EMS and their combination treatments were used in the present investigation. 300 well filled healthy seeds packed in moist germination paper were selected for each treatment in the gamma chamber at 100, 200, 300 and

400 Gy doses of gamma rays in 60^{Co} gamma source (irradiation source capacity to release 3000 Ci delivery 7200 r/min). The gamma irradiation installed at Government Institute of Science, Aurangabad (M.S.). India. Similarly, in case of EMS treatment individually and in combination with gamma rays has done. 300 healthy seeds each were presoaked in distilled water for 6 hours at room temperature. For EMS treatment, the presoaked seeds were treated with 5, 10, 15 and 20 mM freshly prepared solution of EMS for 3 hours. For combination treatments 300 seeds each were first irradiated with gamma rays at 100, 200, 300 and 400 Gy doses and then followed by EMS, only one concentration of EMS (10mM) was used in combination with 100, 200, 300 and 400 Gy gamma rays. After the EMS treatment, the treated seeds were washed thoroughly for 1h in running tap water to terminate the residual effect of the mutagenic chemicals. After the completion of the treatment the treated seeds were sown immediately in the field along with their respective controls to rise the M₁ generation in a completely randomized block design (CRBD) with three replications. All the treatments including the controls were raised adopting a spacing of 30 cm in between rows and 15 cm in between plants. All the recommended cultural measures namely, irrigation, weeding and plant production methods were carried out during the growth period of the crop. The M₂ seedlings were screened from 30th to 40th day to record the various chlorophyll mutants periodically. The classification and identification of the chlorophyll mutants was done based on the nomenclature adopted by Gustafson (1940). The mutation frequency was the mutation frequency was estimated on M₂ seedling basis.

Results and Discussion

Frequency of chlorophyll mutations

Leaf color mutations are one kind of most frequently observed mutation in both spontaneous and induced mutant populations, and often used as an indicator of mutagenic effects and efficiency of various mutagens. Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromere and proximal segment of chromosomes (Swaminathan, 1964). Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in various pulse crops by several workers including Gautam *et al.* (1992).

The frequency of chlorophyll mutants in M₂ generation is mainly used as a dependable measure of genetic effects in mutagens (Nilan and Konzak, 1961). On the seedling basis of M₂ generation, progressive increase in the frequency of chlorophyll mutation was observed with increase in all mutagenic dose or concentration. Albina- These mutant leaves were white in colour, due to absence of all pigment. This was led to the death of the plants at 10-15 days after germination. Xantha- The leaves turned yellow in colour due to the absence of xanthophylls. Viridis- These mutants showed leaf margin more segregated at compared to control. Young leaves were pale green in during maturity time. One or two mutants were observed at all mutagenic treatments. Chlorophyll mutations were found in almost all the mutagenic treatments. High frequency of chlorophyll mutations were found in the

gamma rays, EMS and combination treatment of gamma rays with EMS. The highest frequency of chlorophyll mutations (2.93%) was reported in the (400 Gy Gamma Rays + 10mM EMS), while the lowest (1.16%) frequency of chlorophyll mutations was found in 100 Gy Gamma Rays. I was found Combination of Gamma Rays and EMS to be more effective for inducing chlorophyll mutations in comparison to Gamma rays and EMS (Table-1). Among all the mutagens tested EMS induced maximum frequency of chlorophyll mutations indicating their greater effectiveness. Higher frequency and spectrum of chlorophyll mutations suggest that the chemical mutagen EMS is more efficient in inducing mutations of genes needed for chlorophyll development (Shah *et al.*, 2006). Higher frequency and a wider spectrum of chlorophyll mutants in chemical mutagen EMS have been reported by Bhattacharya (2003), Sharma and Sharma (1984), Marki and Bianu (1970), Kawai and Sato (1969) in carnation, lentil, flax and rice, respectively.

Spectrum of chlorophyll mutations

The spectrum of chlorophyll mutations obtained in the present study induced different types, viz., Albina, Xantha and Viridis was observed. These types mutations observed in Grasspea M₂ generations.

Chlorophyll mutants

Albina

These seedlings were characterized by their dull white color and were devoid of

Table.1 Effect of different combination of gamma rays and EMS on frequency and spectrum of chlorophyll mutations in M2 generation of Grasspea

Treatments(Dose/Conc.)		Number of Plant Studied	Spectrum of chlorophyll mutant			Total No.of Chlorophyll Mutant	% of Mutation Frequency
			Albino	Xantha	Viridis		
Gamma Rays	100 Gy	430	1	2	2	5	1.16
	200 Gy	428	1	4	2	7	1.63
	300 Gy	400	1	4	3	8	2
	400 Gy	380	3	5	2	10	2.63
EMS	5 mM	425	2	2	2	6	1.41
	10 mM	418	2	3	3	8	1.91
	15mM	385	3	4	5	9	2.33
	20 mM	370	4	6	3	10	2.70
Combination(Gamma Rays +EMS)	100Gy+10mM EMS	405	3	4	4	8	1.97
	200Gy+10mM EMS	390	4	5	4	9	2.30
	300Gy+10mM EMS	370	4	6	4	10	2.70
	400Gy+10mM EMS	375	7	5	5	11	2.93

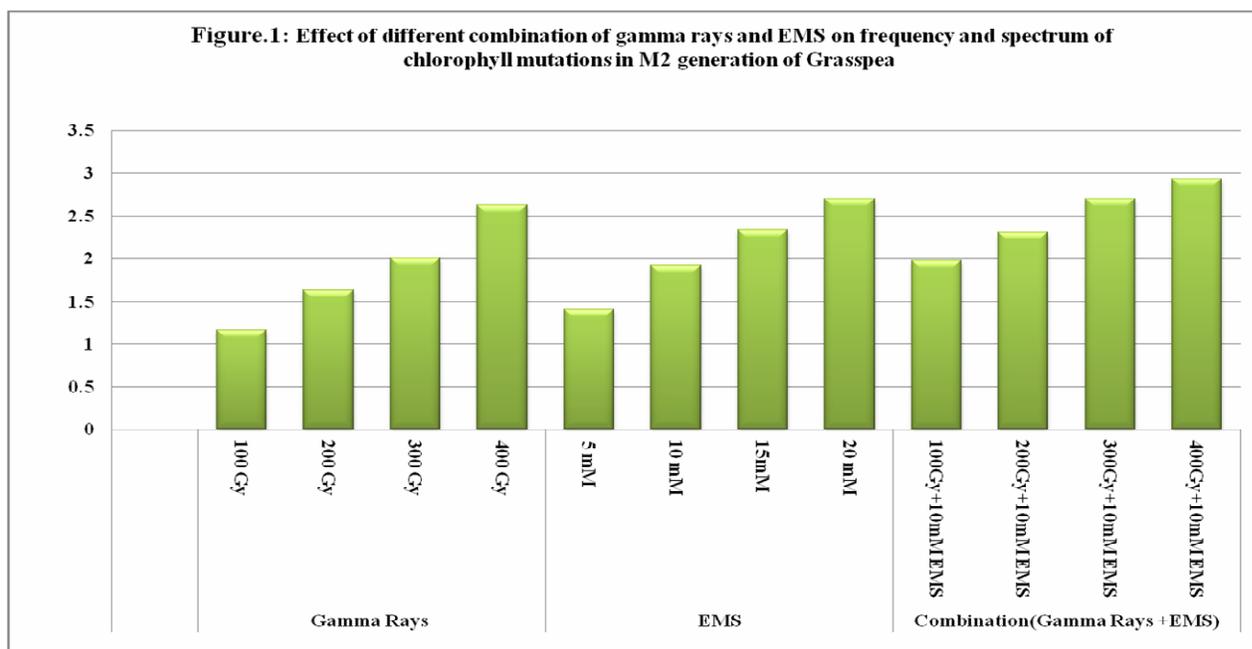


Figure.1 Albina



Figure.2 Xantha



Figure.3 Viridis



chlorophyll, carotenoide and other pigments. Albino seedlings are smaller in height and survive to a maximum of 20 days after germination and then die.

Xantha

Xantha mutants were yellow in colour. These mutants survived for 30- 35 days and became stunted in growth.

Viridis

The seedlings are dark green in the early stages of development and turn normal

green in the later stages. The mutants produce normal looking flowers and also set seeds. These seedlings survived up to 40-45 days.

It concluded that viable chlorophyll mutations, i.e., viridis were produced more at higher doses/concentrations of mutagens whereas lethal mutants, namely, albina, xantha were observed more frequently at relatively low doses/concentrations of the mutagens. The chlorophyll mutations do not have any economic value due to their lethal scenery

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.

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