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Induction of chlorophyll mutation through physical and chemical mutagen in *Phaseolus lunatus* (Lima Bean)

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ABSTRACT

The present investigation was undertaken to study the frequency of chlorophyll deficient sectors and chlorophyll mutants induced by Gamma rays at the dosage of 240Gy,300Gy,360Gy,420Gy, Ethyl Methene Sulphate (EMS) at the concentrations of 0.25%,0.50%,0.75%,1% and the Combination of both (Gamma rays and EMS) like 240Gy+1%, 300Gy+0.75%, 360Gy+0.50%, 420Gy+0.25% in the variety 'King of Garden' *Phaseolus lunatus* (lima bean).The chlorophyll mutants were observed in all the three mutagenic treatments in the lima bean. These mutants were White (*Albina*), Yellow (*Xantha*), Yellow green (*Chlorina*) and Light green (*Viridis*). They were screened after 10-15 days of old seedling and were found at the margin of the leaflet or the entire leaflet.Induced mutation in the sectors of the leaves resulted into chlorophyll chimeric plants.

The scoring of chlorophyll mutation frequency in M_2 generation was one of the most reliable measures for evaluating the mutagenic induced genetic altercation of the mutagen treatments used in the ideotype. The frequency of chlorophyll mutants increased with the increase in the dose/concentration of the mutants. They can be screened and enhanced through mutation breeding for enormous yield.

Figures : 03 References : 13 Tables : 02
KEY WORDS : Chimera, Chlorophyll mutants, EMS, Gamma rays, Lima bean

Introduction

Phaseolus lunatus variety King of Garden belongs to family Fabaceae. It is also called as lima bean, butter bean, double bean *etc.* which predominantly is a self-fertilizing plant. It is an ancient crop which is widely distributed in the tropical countries like India. It is most drought resistant crop that can grow in diverse environmental condition. Lima bean is an annual and perennial crop which was propagated through its seeds. This is herbaceous, twining climber crop. The vegetative period of lima bean is in the range of 80 days-90 days or 120 days. The leaves are alternate and tri-foliate, ovate to lanceolate leaflets. Inflorescences are 2cm long and produce white to yellow or violet bisexual flowers which are less than 1 cm in length.

The fruits are 3.5-12 cm long, dehiscent pods with 2 to 4 seeds. Seeds vary in shape, size and color. The seeds are mostly kidney shaped, which are 1.50 cm, long, flat and thin, white, creamy, red or speckled. The stem may be upto 4.5-8m long. It has thin tap root system. Lima bean is a minor grain legume and produces upto 2000 to 8000 kg of fresh seeds which depend on cultivar type and cultivation conditions. In the tropics, in experimental conditions, climbing types grown in pure stands may yield 750kg/hector dry seeds whereas bushy types may yield 2000-2500 kg/hector.

Material and Methods

Collection of Genotype:

The Experimental genotype selected for the present investigation was *Phaseolus lunatus* variety King of Garden. It is commonly known as Double bean in Marathi. The experimental seed material was collected from Sheti Udyog Bhandar, Swargate, Pune, Maharashtra, India.

Mutagens Used: Physical mutagen gamma rays, chemical mutagen EMS and combination of both

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TABLE -1 : Effect of mutagens on the spectrum of Chlorophyll mutants in M_2 generation of *Phaseolus lunatus*.

Mutagens	Dose/Concentration	Frequency of Chlorophyll mutants				
		Albina	*Xantha*	*Chlorina*	*Viridis*	Total
Control		-	-	-	-	-
Gamma Rays	240Gy	-	1	1	2	4
	300Gy	2	1	1	1	5
	360Gy	1	2	2	1	6
	420Gy	1	3	1	3	8
EMS	0.25%	-	1	1	1	3
	0.50%	1	1	2	1	5
	0.75%	2	1	1	1	5
	1%	1	2	2	1	6
Gamma Rays+ EMS	240Gy+1%	1	1	1	1	4
	300Gy+0.75%	1	1	2	1	5
	360Gy+0.50%	1	2	-	2	5
	420Gy+0.25%	2	1	1	3	7

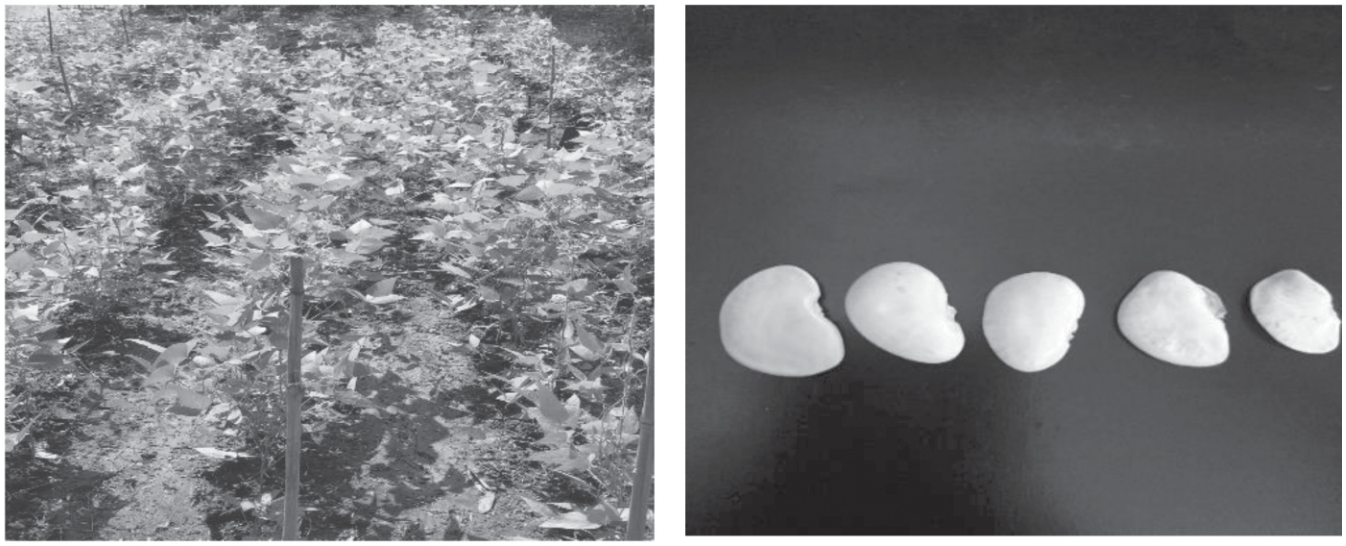

Fig. 1- *Phaseolus lunatus* (Lima bean)

mutagens were used for the treatment.

1.Gamma Rays Treatment: Healthy, uniform size and dry seeds of *Phaseolus lunatus* L. variety King of Garden (Pole) were packed in the polythene bags and sealed for

TABLE -2 : Effect of Mutagens on the frequency of chlorophyll mutants in M_2Generation of *Phaseolus lunatus* L.

Mutagens	Dose/Concentration	Frequency of Chlorophyll mutants				
		Albina	***Xantha***	***Chlorina***	***Viridis***	**Total**
Control		-	-	-	-	-
Gamma Rays	240Gy	-	2.083	2.083	4.166	8.33
	300Gy	4.347	2.173	2.173	2.173	10.86
	360Gy	2.222	4.444	4.444	2.222	13.33
	420Gy	2.325	6.976	2.325	6.976	18.6
EMS	0.25%	-	2.222	2.222	2.222	6.66
	0.50%	2.38	2.38	4.761	2.38	11.9
	0.75%	5.128	2.564	2.564	2.564	12.82
	1%	2.564	5.128	5.128	2.564	15.38
Gamma Rays+ EMS	240Gy+1%	2.5	2.5	2.5	2.5	10
	300Gy+0.75%	2.564	2.564	5.128	2.564	12.82
	360Gy+0.50%	2.702	5.405	-	5.405	13.51
	420Gy+0.25%	5.405	2.702	2.702	8.108	18.91

Gamma radiation. Electromagnetic ionizing radiations were applied from CO^{60} source of irradiation. The seed samples were exposed to different doses of Gamma rays like 240Gy, 300Gy, 360Gy and 420Gy. Gamma radiation was carried out at Nuclear Chemistry Division, Department of Chemistry, Savitri Bai Phule University of Pune, Ganeshkhind, Pune -411007.

2.EMS: Ethyl Methene Sulphonate (EMS) was obtained from Spectrochem. Pvt. Ltd. Mumbai (India) with a molecular weight 124.16 g/mol and its density 1.20g/$cm^{3.}$This experimental research work was to determine the lethal doses of LD_{50} at suitable concentration of mutagens. EMS treatments like 0.25%, 0.50%, 0.75% and 1% were administered at room temperature.

3.Combination: For the combination treatment, Gamma rays irradiated seeds were used. After the physical mutagenic treatment, chemical mutagenic treatment of EMS was conducted on the seed samples. In combination treatment Gamma rays and EMS mutagens were used like 240Gy+1%, 300Gy+0.75%, 360Gy+0.50% and 420Gy+0.25%.

Experimantal Observation

1.Chlorophyll mutations (Table No. 1)

The M_2 generation was raised from the harvested and collected seeds of the M_1 generation plants. The plants were screened after 10-15 days old seedling. The chlorophyll mutants like, *Albina, Xantha, Chlorina and Viridis* were recorded in *Phaseolus lunatus* L. of M_2 plants.

Following were the characteristics of chlorophyll mutations-

a) Albina: *Albina* mutant was completely white in color, such seedling cannot survive more than 10-15 days after seed germination.

b) *Xantha:* *Xantha* mutant was yellow in color, this mutant survived for 20-25 days and growth of the seedling was stunted further.

c) *Chlorina*: *Chlorina* mutant was yellowish green in color, few of them again reverted to normal green color and they survived upto 40-45 days

d) *Viridis:* *Viridis* mutant was light green in color and the seedling could survive up to 50-55 days.

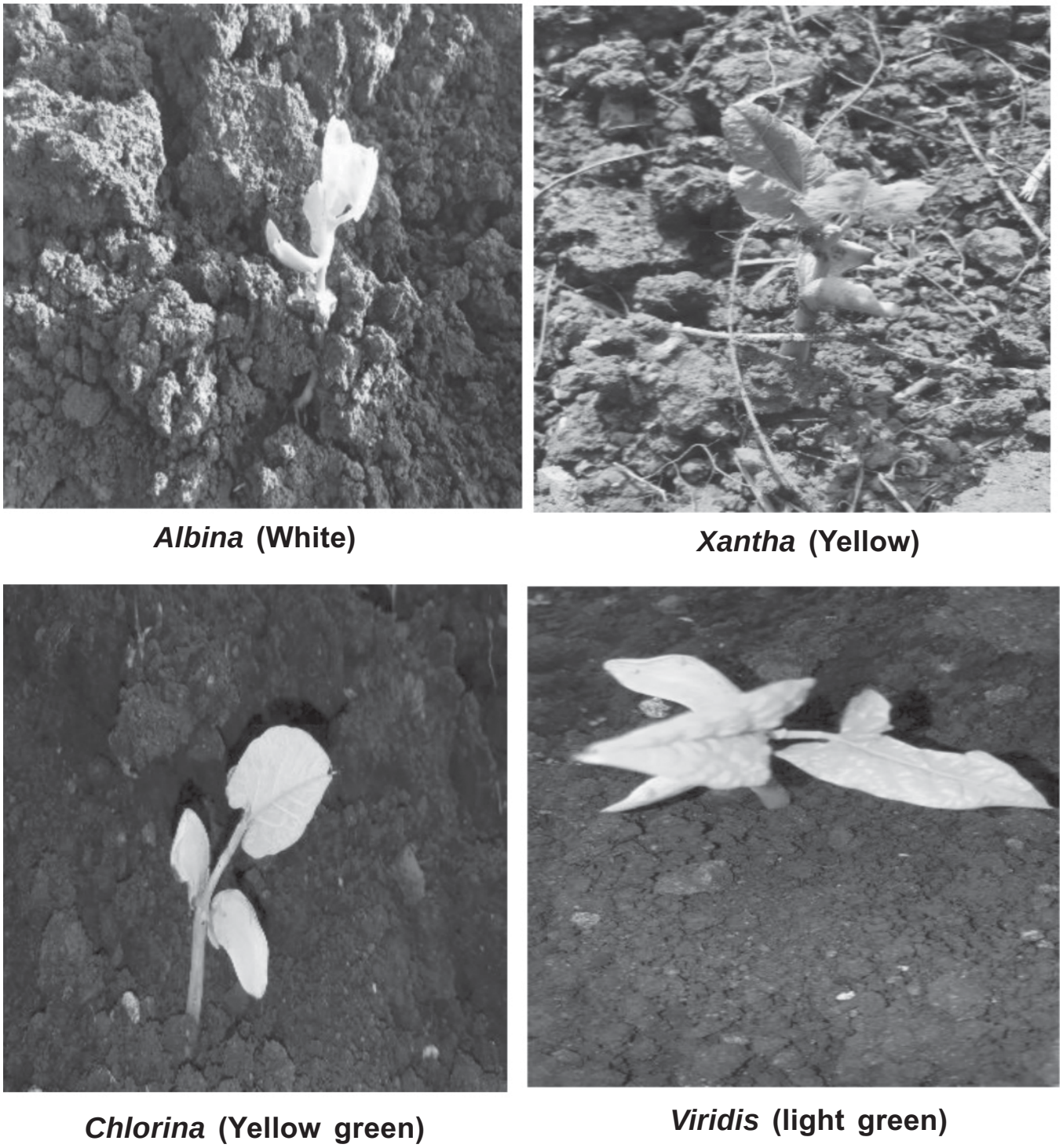

Fig. 2 : Chlorophyll mutation in *Phaseolus lunatus*.

Albina (White), *Xantha* (Yellow), *Chlorina* (Yellow green), *Viridis* (light green)

In the present investigation the frequency of the chlorophyll mutants in Gamma rays treatment was in the range of 8.33-18.60%, in EMS it was 6.66-15.38% and in Combination treatment the frequency of the chlorophyll mutant was 10.00-18.91%.The highest frequency of the chlorophyll mutants in Gamma rays 18.60% was observed at 420Gy and lowest 8.33% was observed at 240Gy.In EMS the highest frequency percentage of chlorophyll mutants 15.38 % were recorded in the 1% EMS and lowest 6.66% at 0.25% EMS. In Combination treatment the highest frequency of chlorophyll mutants was observed to be 18.91% at 420Gy+0.25%

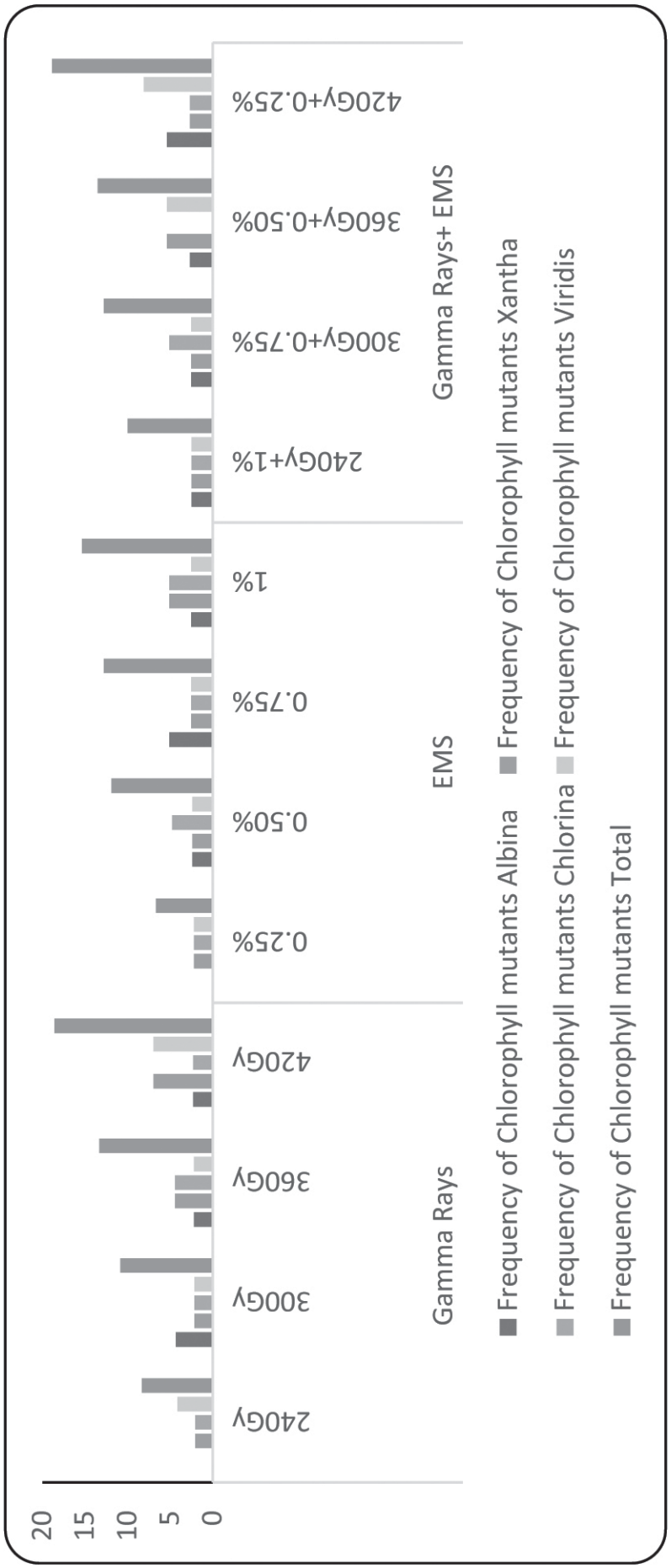

Fig.3 : Effect of Mutagens on chlorophyll mutants in M_2Generation of *Phaseolus lunatus* L.

concentration and lowest 10.00% at 240Gy+1% treatment. Amongst all the three mutagenic treatments the highest frequency of chlorophyll mutant was 18.91% observed in 420Gy+0.25% treatment and lowest was 6.66% at 0.25% EMS treatment.

Result and Discussion

1. Chlorophyll mutation: The scoring of chlorophyll mutation frequency in M_2 generation was one of the most reliable measures for evaluating the mutagenic induced genetic altercation of the mutagen treatments used in the ideotype. Enhancement of mutation frequency and alteration of mutation spectrum are two important goals of mutation[9]. The chlorophyll mutants were classified into *Albina*, *Xantha*, *Chlorina* and*Viridis*. It was pointed out that chemical mutation showed higher frequency of chlorophyll mutation than radiations like Gamma rays[5,6]. The frequency of the chlorophyll mutants was increased with the increase in dose/concentration of the mutagens. Worker[11] recorded the increase in frequency of chlorophyll mutation as the dose/conc increases of Gamma radiation in cluster bean variety NCB-12.The frequency of the chlorophyll mutants was increased with the increase in the dose and concentration of the mutagens reported[7]. There were increases in the spectrum and frequency of chlorophyll mutation used singly or in combination. Chlorophyll mutation was caused by many factors such as non-functional chlorophyll biosynthesis or lack of carotenoids. The highest frequency of chlorophyll mutants was 18.91% observed in 420Gy+0.25% treatment and lowest was 6.66% at 0.25% EMS treatment in *Phaseolus lunatus* L. An investigator[9] observed the linearity in dose or the concentration that took place due to chlorophyll mutations. The combined treatment of Gamma rays and EMS showed higher frequencies than the individual

treatments in *Vigna radiata* (L.)[2]. Frequency of chlorophyll mutations was increased with increasing doses/ concentrations of both single and combined treatments[8]. Workers[4] reported that varietal differences and variations in incidence of chlorophyll mutations is due to the differences in the number of genes controlling the chlorophyll development in different varieties. Such varietal differences have also been reported earlier[1]. Furthermore, Cluster bean variety Pusa- Navbahar showed a dose dependency, where low doses of Gamma rays1.69% have highest mutation frequency and 0.89% have lowest mutation frequency at higher doses of Gamma rays[13]. Similar results were observed earlier in lima bean[10].

Conclusion

Physical availability and economic accessibility of food is very important for the food security.Induced mutation in various plants is a great way to increase the world food supplies. Plant breeding programs carried out in various ways have successfully evolved in many genetically improved variants of pulses and vegetable crops.The mutation varieties showed differential response of genotypes due to the genetic makeup in the induction of chlorophyll mutations. Plants can be screened and enhanced for higher yield through mutation breeding programs. Hence mutation breeding is an effective substitute to conventional breeding.

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