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EFFECT OF EMS AND GAMMA RAYS INDUCED CHLOROPHYLL MUTATION IN SOYBEAN *(GLYCINE MAX* L.)

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In the present investigation, attempts were made to induce genetic variability of two cultivars of soybean *(Glycine. max* L) JS-335 and DS-228 by employing physical (Gamma rays) and chemical (Ethyl Methane Sulphonate) mutagens. The germplasm of two cultivars were procured from M.P.K.V. Rahuri, Dist-Ahmednagar. The uniform seeds of two cultivars were treated with four different concentration of EMS (0.05, 0.10, 0.15, and 0.20%) for 12hr and 18hr, respectively. Dry seeds with for different doses of Gamma rays 5KR, 10KR, 15KR and 20KR. The two hundred treated seeds of each concentration / dose with untreated seeds were sown in the field during kharif of 2010 in RBD (25x50cm). The parameters, percentage seed germination, pollen sterility, survival of plants, seedling injury were studied in M_1 generation. M_2 population was screened for the frequency and spectrum of chlorophyll mutations. Gamma ray was found highly effective to induce high frequency of chlorophyll mutations like Xantha, Chlorina and Albino as compared to EMS in cultivar JS-335 but in cultivar DS-228 frequency chlorophyll mutations was more in EMS treatments than the Gamma rays treatments. Out of these Chlorina was predominate in both mutagenic treatments over the Albino and Xantha.

Keywords : Chlorophyll mutation; EMS; Gamma Rays; Mutagenic sensitivity; Soybean.

Introduction

Soybean (*Glycine max* L) is one of the important miracle crops with about 40% protein and 20% oil content. It belongs to family Fabaceae.Soybean originated in China and is being cultivated there for more than 4000 years[1]. Soybean has new become the largest source of vegetable oil and protein in the world and about 95% of the worlds annual soybean production is from major countries like USA, Brazil, Argentina, China and India. 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 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[2-6]. In the present study the effect of gamma rays and Ethyl methane sulphonate (EMS) applied single as well as in combination on the frequency and spectrum of chlorophyll mutation was studies in two cultivar of soybean.

Material and Method

Seeds of two soybean cultivars, DS-228 and JS 335 used in the present investigation were procured from Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharashtra) both the cultivars are widely cultivated in Maharashtra. To begin with pilot experiments were conducted to determine the lethal dose(LD_{50}), suitable concentration of the mutagens and duration of treatment for both the cultivars of soybean. Both the varieties of soybean were treated separately with chemical EMS and physical mutagen (Gamma radiation). From such experiments it was finally established that concentration of 0.05, 10, 15 and 20mM for duration of 12 hrs and 18hrs hours are best suitable for mutagenic treatments for the cultivars of soybean for chemical mutagen treatments. Seeds were presoaked in distilled water for 6 hours and subjected to freshly prepared mutagen solutions for 12 hrs and 18 hrs at 25± 2°C with intermediate shaking. The volume of mutagenic solutions was about 5 times to that of seeds. The seeds, treated with chemical mutagens were thoroughly washed under running tap water for an hour to terminate the reaction of the chemical.

For physical mutagen treatment, dry seeds with a moisture content of 10-12% were irradiated with 5KR, 10KR, 15KR and 20KR from a CO^{60} source available in the Department of Biophysics, Government Institute of

Table 1. Differential effects of mutagens on the frequency of chlorophyll mutation in M_2 generation of varaity-DS-228 in soybean.

Treatments	Concentration (%)	No. of plant observed	No. of chlorophyll mutants scored	Frequency of chlorophyll mutants	Relative% of chlorophyll mutants			
					Albina	Xantha	Chlorina	Viridis
Control	---------	182	----------	-------	------	----	-----	------
EMS (12hr)	0.05	175	5	2.85	0.57	0.57	1.4	0.57
	0.10	167	7	4.19	1.196	0.598	1.79	0.598
	0.15	160	11	6.87	1.248	0.624	2.88	1.248
	0.20	143	10	6.99	1.99	0.998	4.99	1.99
EMS (18hr)	0.05	144	3	2.08	-------	------	1.386	0.693
	0.10	138	5	3.62	0.724	0.724	1.448	0.724
	0.15	127	7	5.51	1.574	-----	2.152	1.574
	0.20	115	6	5.21	1.73	-----	1.73	1.73
Gamma rays	5KR	171	4	2.33	0.582	0.582	1.164	-----
	10KR	146	7	4.79	0.684	0.684	1.368	0.668
	15KR	130	5	3.84	0.768	0.768	2.304	-----
	20KR	114	4	3.50	0.875	0.875	1.75	------

Table 2. Differential effects of mutagens on the frequency of chlorophyll mutation in M_2 generation of varaity-JS-335 in soybean.

Treatments	Concentration (%)	No. of plant observed	No. of chlorophyll mutants scored	Frequency of chlorophyll mutants	Relative% of chlorophyll mutants			
					Albina	Xantha	Chlorina	Viridis
Control	---------	183	----------	-------	------	----	-----	------
EMS (12hr)	0.05	173	4	2.31	0.577	0.577	1.15	------
	0.10	145	7	4.82	0.688	0.688	2.065	1.337
	0.15	135	7	5.18	2.22	1.48	1.48	-------
	0.20	121	5	4.13	0.826	0.826	1.65	0.826
EMS (18hr)	0.05	139	2	1.43	0.715	------	0.715	-----
	0.10	129	4	3.10	0.775	0.775	0.775	0.775
	0.15	117	3	2.56	0.853	------	0.853	0.53
	0.20	105	7	6.66	1.902	1.902	2.854	-----
Gamma rays	5KR	131	6	4.58	1.526	1.526	1.526	1.776
	10KR	122	8	6.55	1.736	1.736	1.736	-----
	15KR	115	6	5.21	0.868	1.736	2.604	-----
	20KR	109	4	3.66	0.915	0.916	0.915	0.915

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Every treatment was carried out for 200 seeds. The treated seeds along with control were sown in the field in randomized block design (RBD) in three replications at spacing of 25 cm in rows and 50 cm between rows to raise M_1 generation during Kharif season of 2010-2011. The individually harvested M_1 plants were sown in the field to raise M_2 generation during Kharif season of 2011-2012 in separate rows. The M_2 progeny was raised along with parental varieties (control) following randomized block design with 3 replication. Each treatment comprised of 20-21 M_1 plant progenies and each M_2 progeny row consisted of 10 to 25 plants in three replications. The cultural operation and application of FYM were done as per schedule. The frequency of chlorophyll mutation was scored through the life span of

the M_2 progeny. Chlorophyll mutation was calculated as the percentage of mutated plants.

Results and Discussion

In the present investigation total 4 types of chlorophyll mutations as Xantha, Chlorina, Albino and Viridis were recorded in two cultivar of soybean. The frequency and spectrum of chlorophyll mutant were represented in the Table 1 and 2.

The spectrum of chlorophyll mutation was large. Result revealed that only Chlorina chlorophyll mutant was recorded in all the treatments of the both mutagen of two cultivars. The total frequency of chlorophyll mutants observed was 6.99 (0.20% EMS-KR) and it was maximum in the cultivar DS-228. Twenty five percent of total chlorophyll mutation were of Chlorina type and the frequency was maximum in EMS treatment (0.20% for 18 hrs) in JS-335. The maximum frequency of chlorophyll mutants observed were 6.66 in 0.020% EMS for 18 hrs in JS-335. Treatment (0.20% EMS of 18 hrs.) 20 to 25% percent of total chlorophyll mutations were of Chlorina type and the remaining were Albino and Xantha.

The presence of absence of chlorophyll mutants in some mutagenic treatment is directing differences in the availability of mutagenic loci to the mutagen. The frequency of chlorophyll mutants was higher in Gamma rays than the EMS. Increase in the frequencies of chlorophyll mutation with increase in the concentration / dose reported by Arvind Kumar *et al.*[7], Satpute and Kothekar[8] and Solanki and Sharma[9].

The results obtained are confirming that the gamma ray is efficient mutagen than the EMS to induced maximum chlorophyll mutation. The order of chlorophyll mutant in soybean was Chlorina,Albino soybean and Xantha.

In the present Investigation, the induction of high frequency of chlorophyll mutations may be attributed to the method of treatment and efficiently scoring and handling of mutaginized populations. It is therefore concluded that although the chlorophyll mutation do not have any economic value due to their lethal nature, such a study could be useful in indentifying the threshold dose of a mutagen that would increase the genetic variability and number of economically useful mutant in the segregating generations.

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