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CHLOROPHYLL MUTATIONS IN *VIGNA RADIATA* (L.) WILCZEK. II. MUTAGENIC EFFECTIVENESS AND EFFICIENCY OF CHEMICAL MUTAGENS

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Abstract

Using EMS, MMS and SA, mutations were induced in two mungbean (*Vigna radiata* (L.) Wilczek) varieties viz., PS-16 and Pusa Baisakhi. Three different types of chlorophyll mutants viz., albina, *Chlorina* and *Viridis* were observed in M_2 generation. Chlorophyll mutation frequency increased with increase in concentration of various mutagens. EMS produced highest frequency of mutations followed by MMS and SA. On the basis of their effectiveness, three mutagens stood in the declining order of MMSSAEMS whereas on the basis of their efficiency, the sequence was EMS, MMS and SA. All the three mutagens were found to be effective and efficient at the lower mutagen concentrations.

Introduction

During past few years intensive research work has been initiated to improve yield as well as the quality of mungbean (*Vigna radiata* (L.) Wilczek). For the genetic improvement of mungbean it is essential to provide additional variability and to monitor this variability, it is necessary to provide information on the effectiveness and efficacy of various mutagens. The present investigations provide experimental data on the mutation frequency and spectrum in mungbean with particular reference to the mutagenic effectiveness and efficiency of ethylmethane sulphonate (EMS), methylmethane sulphonate (MMS) and sodium azide (SA).

Materials and Methods

Two varieties of *Vigna radiata* (L.) Wilczek viz., PS-16 and Pusa Baisakhi were selected for treatment with chemical mutagens. Atleast 250 seeds of uniform size presoaked in distilled water for 9 h were treated with freshly prepared solution for 6 h with intermittent shaking at $27 \pm 1^{\circ}C$. Seeds were thoroughly washed in running tap water to reduce the residual effect of the mutagens sticking to the seed coat. The treated seeds were sown in field to raise M_1 population and each M_1 plant was harvested separately and sown as progeny rows to raise M_2 population. Ethylmethane sulphonate (EMS), methylmethane sulphonate (MMS) and sodium azide (SA) were used as chemical mutagens. Frequency and spectrum of chlorophyll mutations were scored at seedling stage on the basis of M_2 plant progeny and M_2 population in treated and control population. Chlorophyll mutants were identified and classified according to Gustaffson (1940) and Siddiqui (1973). Formulae suggested by Konzak *et al.*, (1965) were used to evaluate mutagenic effectiveness and efficiency of the mutagens used.

Table 1. Frequency and spectrum of chlorophyll mutants in M_2 generation of mungbean.

Treatment	Var. PS-16				Frèquency (%)	Var. Pusa Baisakhi				Frequency (%)
	% mutated plant progenies (Mp)	Chlorophyll mutant types Albina	Chlorina	Viridis		% mutated plant progenies (Mp)	Chlorophyll mutant types Albina	Chlorina	Viridis	
Control	-	-	-	-	-	-	-	-	-	-
0.1% EMS	56.52	2	13	05	12.68	57.69	5	15	07	12.50
0.2% EMS	57.69	4	17	08	16.51	63.33	8	22	09	19.43
0.3% EMS	61.90	3	18	06	21.82	76.00	4	20	11	22.94
0.4% EMS	64.28	6	26	10	22.04	78.57	7	27	13	23.65
0.01% MMS	42.85	2	10	02	09.52	40.74	3	13	01	08.33
0.02% MMS	53.57	3	16	04	14.41	56.00	5	20	03	15.00
0.03% MMS	54.54	3	17	03	18.24	57.14	2	21	03	15.17
0.04% MMS	66.67	6	25	07	21.10	60.86	6	25	06	23.77
0.01% SA	26.92	2	-	-	03.69	28.00	-	06	04	06.19
0.02% SA	27.27	1	-	-	01.00	29.62	-	08	02	07.83
0.03% SA	37.50	3	-	-	04.67	34.78	-	05	04	08.30
0.04% SA	40.74	4	-	-	06.96	40.00	-	09	03	08.92

Results

Chlorophyll mutations: Chlorophyll mutations were recorded in the field in M_2 generation when seedlings were 7 to 15 days old. The spectrum of different M_2 chlorophyll mutants included, albina, chlorina and viridis in the two varieties of mungbean viz., PS-16 and Pusa Baisakhi after treatment with different chemical mutagens. The seedlings which initially looked to be normal started showing different types of chlorophyll defects at the later stage of growth. All these chlorophyll deficient mutants were lethal except viridis which produced 4-6 g seeds in comparison to 9-10 g in control plants. A brief description of the different chlorophyll mutants is given below:

Albina: The seedlings survived for about 8 to 10 days after germination.

Chlorina: Most of these seedlings died within 15 days. However, 5% plants possessed vigour and were 3 days late in flowering and maturity as compared to control.

Viridis: Leaf size was reduced but leaf shape remained unaltered. The plants were slow growing and had a reduced size and a low seed yield. The mutant was distinguished because of its reduced height and viridine green colour of leaves.

Frequency and spectrum of chlorophyll mutations: The chlorophyll mutation frequency was calculated on progeny basis as well as on plant basis (Table 1). The trend of the mutation frequency was similar in both the methods. Although response to chemical mutagens in different varieties differed in relation to frequency of chlorophyll mutations, var. Pusa Baisakhi appeared to produce more chlorophyll mutants than var. PS-16. The frequency of chlorina mutants was the highest in both the varieties, followed by those of viridis and albina. The var. PS-16 produced no viridis and chlorina type with SA treatments. On the contrary, albina was not observed in the var. Pusa Baisakhi with the same treatments. The frequency of chlorophyll mutations seems to be concentration dependent and increases

Table 2. Effectiveness and efficiency of different mutagens in M_2 generation of mungbean.

Treatment	Var. PS-16			Var. Pusa Baisakhi		
	Mutagenic effectiveness Mp/t.c.	Mutagenic efficiency Mp/I	Mp/S	Mutagenic effectiveness Mp/t.c.	Mutagenic efficiency Mp/I	Mp/S
0.1% EMS	094.20	2.81	4.17	096.15	4.41	4.12
0.2% EMS	048.07	2.57	3.10	052.77	2.46	3.34
0.3% EMS	034.38	1.56	2.69	042.22	2.42	3.32
0.4% EMS	026.78	1.24	2.05	032.73	1.43	2.56
0.01% MMS	714.16	2.84	3.34	679.00	3.37	3.25
0.02% MMS	446.41	1.83	2.96	466.66	2.41	3.16
0.03% MMS	303.00	1.54	2.54	317.44	1.74	2.71
0.04% MMS	277.79	1.17	2.37	253.58	1.21	2.04
0.01% SA	448.66	0.99	2.36	466.66	1.57	2.62
0.02% SA	227.25	0.83	1.86	246.83	1.33	2.03
0.03% SA	208.33	0.69	1.96	193.22	0.68	1.85
0.04% SA	169.75	0.57	1.57	166.66	0.55	1.62

with concentration of the mutagens (Table 1). EMS induced the highest frequency of chlorophyll mutations followed by MMS and SA in both the varieties.

Mutagenic effectiveness and efficiency: The mutagenic effectiveness as measured by the percentage of chlorophyll mutations divided by the dose of the mutagen was as high 714. 16 with 0.01% MMS in the var. PS-16 (Table 2), while the highest effectiveness of EMS and SA treatments was 96.15 and 679.00, respectively, in the var. Pusa Baisakhi. The effectiveness decreased with increase in concentration of mutagens in all the cases. The order of mutagens based upon effectiveness was MMS, SA and EMS.

The mutagenic efficiency varied depending on the criteria selected for its estimation. In the present study, the mutagenic efficiency, worked out on the basis of seedling injury (Mp/I) and pollen sterility (Mp/S), showed a decline with increasing concentrations in all the treatments (Table 2). The mutagenic efficiency, as calculated by the relation (Mp/I), gave 2.045, 1.845 and 0.770 values for EMS, MMS and SA treatments, respectively, in the var. PS-16 whereas in the var. Pusa Baisakhi, the efficiency values were 2.680, 2.182 and 1.032 (Table 3). The mutagenic efficiency, as measured by the percentage of mutated progenies divided by sterility (Mp/S), was the highest in EMS treated populations and the lowest in SA (Table 3). In general, the efficiency of mutagens in a descending order was EMS, MMS, SA. The efficiency calculated on the basis of sterility was higher as compared to seedling injury. On the basis of seedling injury and sterility, the EMS was found to be more efficient and SA was less efficient in both the varieties in question.

Discussion

The chlorophyll mutation frequency is useful in assessing the potency of a mutagen. Hence, scoring of chlorophyll mutations has proved to be a much dependable index for evaluating the genetic effects of the mutagenic treatments. A comparison of chlorophyll mutations indicates that the frequency of chlorophyll mutations recorded in M_2 generation was concomitant with dose. Similar dose dependent increase in the chlorophyll mutations frequency was reported by Gaul (1964) in barley, Blixt *et al.*, (1966) in peas, Nerker (1976) in *Lathyrus sativus* and Khan & Siddiqui (1990) in *Vigna radiata*. However, Khan (1979) suggested that the frequency of chlorophyll mutations in mungbean is dose independent.

The present investigation revealed that EMS induced the highest frequency of chlorophyll mutants in both the varieties of mungbean, EMS induced chlorophyll

Table 3. Mutation rate of different mutagens in relation to the biological effects such as injury and sterility in two varieties of mungbean.

Mutagen	Var. PS - 16		Var. Pusa Baisakhi	
	MRI	MRS	MRI	MRS
EMS	2.045	3.002	2.680	3.340
MMS	1.845	2.802	2.182	2.790
SA	0.770	1.940	1.032	2.030

mutants have been reported in peas and *Lens esculenta* (Wellensick, 1965; Uhlik, 1972). Swaminathan *et al.*, (1962) proposed that such a high frequency is due to the preferential action of EMS on chlorophyll development genes located near centromere. EMS is supposed to be specific to certain chromosomal regions (Goud, 1967) containing genes for chlorophyll development and has been reported to induce high frequencies of chlorophyll mutations (Natrajan & Upadhya, 1964).

The frequency of chlorophyll mutations induced by SA was much less as compared to EMS and MMS. The low chlorophyll deficient mutation frequency may be due to the inhibition of catalase and peroxidase and the increase in peroxide concentration in the cell (Kleinhofs *et al.*, 1978). Nilan *et al.*, (1973) observed that the greater effectivenss of azide in the acid form is probably due to a better penetration of the cell membranes by the neutral NH_3 molecules.

Mutagenic effectiveness is an index of the response of a genotype to the increasing dose of the mutagen. The order of the mutagenic effectiveness as determined on the basis of mutated plant progenies was MMS, SA and EMS. All the three chemical mutagens were found to be more effective at lower concentrations. The decline in the mutagenic effectiveness recorded at higher doses shows that the increase in mutation rate was not proportional to the increase in the doses of various mutagens. Similar results were obtained by Prasad (1972) for *Triticum*, Nerker (1977) for *Lathyrus sativus*, Farook & Nizam (1978) for *Cicer arietinum* and Khan & Siddiqui (1990) for *Vigna radiata*.

The mutagenic efficiency indicates the extent of genetic damage recorded in M_2 generation in relation to the biological damage caused in M_1. As reported by Ramulu (1970), Sharma (1977) and Khan (1979) the mutagenic efficiency decreased with increasing dose of all the mutagens in our study also. The greater efficiency at lower doses is because the biological damage generally increased with the enhancement in the dose at a higher rate than the mutations yielded in M_2 at the same dose (Konzak *et al.*, 1965).

The lowest efficiency was recorded in SA and the highest in EMS, MMS being intermediate. The results indicate that the efficiency calculated on the basis of sterility was higher as compared with that based on seedling injury. Gaul *et al.*, (1962) reported the mutagenic efficiency to be related to sterility and further observed that the lower the dose, the higher the efficiency. This is because the lower dose may cause relatively less damage, enabling the organism to express the induced point mutations successfully. The sterility induced by chemical mutagens, more particularly by alkylating agents was not found in many cases to be associated with chromosomal abnormalities (Bansal & Natrajan, 1965). Prasad (1968) observed that NMU induced maximum visible mitotic changes in *Triticum durum*, but resulted in a very high degree of sterility. It appears that gene mutations may be responsible for such sterility, although the cytologically undetectable cryptic structural changes may also contribute to some extent. Effectiveness and efficiency of the mutagens differed in both the varieties studied. Goud *et al.*, (1970) and Gupta & Yashvir (1975) reported that the genetic architecture of the organism is a potent factor in determining its response to mutagens.

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