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Efficiency and effectiveness of physical and chemical mutagens in urdbean (*Vigna mungo* (L.) Hepper)

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Abstract: A study was undertaken in a blackgram variety CO 5 to assess the efficiency and effectiveness of physical and chemical mutagen *viz.* gamma ray and EthylMethane Sulphonate (EMS) respectively. Gamma rays were found to be more effective than EMS in producing chlorophyll and viable mutants on M_1 plant and M_2 seedling bases as well as efficient on lethality and sterility bases. Based on injury, EMS was found to be more efficient than gamma rays.

Key words : Efficiency, Effectiveness, Chlorophyll and Viable mutants.

Introduction

A crop plant can be improved in productivity, resistance to biotic and abiotic stresses etc., when the genetic variability for the specific trait is available in the considered population or species. Besides, the inherent variation present in the crop genetic variation can subsequently be made in available in the needed crop plants by recombination breeding. In some cases like pulse crop, due to their autogamous nature and problems of flower drop improvement through hybridization and recombination becomes difficult. Hence, creating variability by mutagenic agents is of paramount importance for pulse crop improvement. Hence, a study was undertaken to assess the efficiency and effectiveness of mutagens in order to find out the suitable mutagen at appropriate dose/concentration so as to use them in applied mutagenesis studies.

Materials and Methods

Seeds of CO5 blackgram (Pureline selection from Musiri local) were subjected to seven different doses of gamma rays ranging from 20 to 80 kR with an interval of 10kR and six different concentrations of Ethyl Methane Sulphonate ranging from 20 to 70mM with 10mM interval. To raise M_1 generation, a total of thirteen treatments along with the control was sown in the field at the rate of 150 seeds for each treatment at a spacing of 30 x 15 cm at Agricultural College and Research Institute, Madurai during June, 1999 in a randomized block design with 3 replications. M_2 generation was raised on M_1 plant basis following plant to progeny method in a randomized block design with 3 replications during January, 2000. Frequency of chlorophyll mutations was calculated per 100 M_1 plant and 100 M_2 seedling bases. The chlorophyll mutants were classified in accordance with the system of Gustaffson (1940) and Blixt and Gottschalk (1975). Frequency of viable mutations was calculated on M_1 plant and M_2 plant bases. Chlorophyll and macromutations were scored treatmentwise to study the mutagenic effectiveness and efficiency. Data on biological abnormalities such as injury, lethality and sterility in M_1 generation and chlorophyll mutation frequencies in M_2 generation were used to determine the mutagenic efficiency and effectiveness according to the formula suggested by Konzak *et al.* (1965).

Mutagenic effectiveness = MP/tc or kR
Mutagenic efficiency = MP/L, MP/I, MP/S

where,

MP = Chlorophyll or viable mutants per 100 M_1 plants; t = Duration of treatment with chemical mutagen in hours; c = Concentration of chemical mutagen in mM; kR = Dose of gamma radiation; L = Percentage of lethality i.e. reduction in survival on 30th day; I = Percentage of injury i.e reduction in plant heght on 30th day; S = Percentage of seed sterility *i.e.* reduction in seed fertility.

Results and Discussion

Frequency

In M_2 generation, a total of 8057 seedlings was scored for chlorophyll mutations selected

Table 1. Frequency of chlorophyll and viable mutants in M_2 generation of blackgram var CO 5

Mutagen (Dose/ Conc)	Frequency of chlorophyll mutants						Frequency of viable mutants					
	No. of M_1 plants		No. of M_2 seedlings		Mutant frequency		No. of M_1 plants		No. of M_2 seedlings		Mutant frequency	
	Scored	Segre-gated	Scored	Segre-gated	Per 100 M_1 plants	Per 100 M_2 seedlings	Scored	Segre-gated	Scored	Segre-gated	Per 100 M_1 plants	Per 100 M_2 plants
Gamma rays (kR)												
Control	15	-	71	-	-	-	15	-	689	-	-	-
20	15	2	700	5	13.33	0.71	15	5	672	14	33.33	2.08
30	15	3	674	8	20.00	1.19	15	4	645	10	26.67	1.55
40	15	3	555	9	20.00	1.62	15	3	508	6	20.00	1.18
50	15	5	575	11	33.33	1.91	15	4	504	5	26.67	0.99
60	15	4	479	6	26.67	1.25	15	5	428	11	33.33	2.57
70	15	1	391	2	6.67	0.51	15	2	347	4	13.33	1.15
80	15	2	627	4	13.33	0.64	15	3	601	8	20.00	1.33
EMS (mM)												
Control	15	-	692	-	-	-	15	-	679	-	-	-
20	15	2	507	4	26.67	0.79	15	4	491	5	26.67	1.02
30	15	3	556	7	20.00	1.26	15	5	519	6	33.33	1.16
40	15	3	310	4	20.00	1.29	15	3	290	4	20.00	1.37
50	15	2	388	2	26.67	0.52	15	2	378	3	13.33	0.79
60	15	2	547	6	26.67	1.10	15	2	530	4	13.33	0.75
70	15	1	341	2	6.67	0.59	15	4	319	7	26.67	2.19

from 225 M_1 plant progenies both for gamma rays and EMS treatments. Percentage of plants segregating for chlorophyll deficiency on the basis of M_1 plant and M_2 seedling bases is furnished in Table 1. Generally chlorophyll mutants occurred in all the doses of gamma rays in different frequencies. In gamma rays doses, the frequency increased from 20 to 50kR on M_1 plant and M_2 seedling bases and thereafter reduction in frequency was observed. Chlorophyll mutation frequency per 100 M_1 plants and M_2 seedling bases was maximum at 50kR. On M_1 plant basis, 20,50 and 60 mM EMS concentrations recorded maximum mutation frequency of 26.67 per cent. On M_2 seedling basis, maximum mutation frequency was observed at 40mM (1.29%). Frequency of chlorophyll mutants was the highest on M_1 plant basis compared to M_2 seedling basis, as recorded by Vanniarajan (1989) and Ahmed John (1997) in blackgram. In this study, the recovery of mutants was less under higher doses of gamma rays and EMS treatments. This might be due to the fact that many of the mutations at higher doses might be eliminated due to lethality. Lower doses of gamma rays were more efficient than higher

Table 2. Mutagenic efficiency and effectiveness in creating chlorophyll mutants on M_1 plant and M_2 seedling bases

Muta- (dose/conc)	Survival reduction on 30th day (Lethality) (L) (%)	Height reduction on 30th day (Injury) (I) %	Seed fertility reduction (Sterility) (S)%	Mutants per 100 M_1 plants (MP)	Mutants per 100 M_2 seedlings (MP)	Effectiveness $\frac{MP \times 100}{tc\ (or)\ kR}$ M_1 plant basis	Effectiveness M_2 seedlings basis	Efficiency M_1 plant basis $\frac{MP \times 100}{L}$	M_1 plant basis $\frac{MP \times 100}{I}$	M_1 plant basis $\frac{MP \times 100}{S}$	M_2 seedling basis $\frac{MP \times 100}{L}$	M_2 seedling basis $\frac{MP \times 100}{I}$	M_2 seedling basis $\frac{MP \times 100}{S}$
Gamma rays (kR)													
20	6.87	23.77	2.45	13.33	0.71	66.65	3.55	194.03	56.08	544.08	10.33	2.99	28.98
30	19.08	29.01	4.76	20.00	1.19	66.67	3.97	104.82	68.94	420.17	6.24	4.10	25.00
40	30.53	31.48	8.19	20.00	1.62	50.00	4.05	65.51	63.53	244.20	5.31	5.15	19.78
50	38.54	30.17	15.40	33.33	1.91	66.66	3.82	86.48	110.47	216.43	4.96	6.33	12.40
60	64.88	30.32	17.57	26.67	1.25	44.45	2.08	41.11	87.96	151.79	1.93	4.12	7.11
70	85.49	50.62	44.65	6.67	0.51	9.53	0.73	7.80	13.18	14.94	0.60	1.01	1.14
80	40.83	13.19	20.90	13.33	0.64	16.66	0.80	32.65	101.06	63.78	1.57	4.85	3.06
EMS (mM)													
20	18.46	27.67	12.11	26.67	0.79	22.23	0.66	144.47	96.39	220.23	4.28	2.86	6.52
30	34.61	18.53	13.60	20.00	1.26	11.11	0.70	57.79	107.93	147.06	3.64	6.80	9.26
40	45.39	1.27	18.76	20.00	1.29	8.33	0.54	44.06	1574.80	106.61	2.84	101.57	6.88
50	32.70	9.22	22.62	26.67	0.52	8.89	0.17	81.56	289.26	117.90	1.59	5.64	2.30
60	40.00	3.80	35.83	26.67	1.10	7.41	0.31	66.68	701.84	74.43	2.75	28.95	3.07
70	48.85	19.28	38.25	6.67	0.59	1.59	0.14	13.65	34.60	17.44	1.21	3.06	1.54

doses of EMS in producing chlorophyll mutations. Khan (1999) gave similar reports in blackgram. According to Ahmed John (1991), the induced genetic changes with low transmission frequency were probably associated with certain minute chromosomal aberra-tions which affected the gene transmission and the mutants were mostly conditioned by single recessive gene.

When the frequency of viable mutants was scored in 7600 M_2 seedlings selected from 225 M_1 plant progenies both for physical and chemical mutagenic treatments, (Table 1) it was maximum at 20 and 60kR gamma ray doses on M_1 plant basis and at 20kR on M_2 seedling basis. In EMS treatments, 30mM recorded maximum viable mutation frequency on M_1 plant basis and 40mM on M_2 plant basis. Frequency of viable mutation on M_1 plant and M_2 plant bases exhibited an inconsistent trend with increase in concentration of EMS (Vanniarajan, 1989. The possible cause of these macromutations may be chromosomal aberrations like small deficiencies or duplications and most probably gene mutations (Singh *et al.* 1980).

Effectiveness

Mutagenic effectiveness of chlorophyll mutations on M_1 plant and M_2 seedling bases is given in Table 2. Maximum and minimum effectiveness of chlorophyll mutations were observed at 30kR (66.67) and 70kR (9.53) respectively on M1 plant basis. On M_2 seedling basis, 40kR recorded maximum

Table 3. Mutagenic efficiency and effectiveness in creating viable mutants on M_1 and M_2 plant bases

Muta- (dose/ conc)	Survival reduction on 30th day (Lethality) (L) (%)	Height reduction on 30th day (Injury) (I) %	Seed fertility reduction (Sterility) (S) %	Mutants per 100 M_1 plants (MP)	Mutants per 100 M_2 seedlings (MP)	Effectiveness $\frac{MP \times 100}{tc \text{ (or) } kR}$: M_1 plant basis	Effectiveness: M_2 seedlings basis	Efficiency, M_1 plant basis: $\frac{MP \times 100}{L}$	Efficiency, M_1 plant basis: $\frac{MP \times 100}{I}$	Efficiency, M_1 plant basis: $\frac{MP \times 100}{S}$	Efficiency, M_2 seedling basis: $\frac{MP \times 100}{L}$	Efficiency, M_2 seedling basis: $\frac{MP \times 100}{I}$	Efficiency, M_2 seedling basis: $\frac{MP \times 100}{S}$
Gamma rays (kR)													
20	6.87	23.77	2.45	33.33	2.08	166.65	10.40	485.15	140.22	1360.41	30.28	8.75	84.90
30	19.08	29.01	4.76	26.67	1.55	88.90	5.17	139.78	91.93	560.29	8.12	5.34	32.56
40	30.53	31.48	8.19	20.00	1.18	50.00	2.95	65.51	63.53	244.20	3.87	3.75	14.41
50	38.54	30.17	15.40	26.67	0.99	53.34	1.98	69.20	88.40	173.18	2.57	3.28	6.43
60	64.88	30.32	17.57	33.33	2.57	55.55	4.28	51.37	109.93	189.70	3.96	8.48	14.63
70	85.49	50.62	44.65	13.33	1.15	19.04	1.64	15.59	26.33	29.85	1.35	2.27	2.58
80	40.83	13.19	20.90	20.00	1.33	25.00	1.66	48.98	151.63	95.69	3.26	10.08	6.36
EMS (mM)													
20	18.46	27.67	12.11	26.67	1.02	22.23	0.85	144.47	96.39	220.23	5.53	3.69	8.42
30	34.61	18.53	13.60	33.33	1.16	18.52	0.64	96.30	179.87	245.07	3.35	6.26	8.53
40	45.39	1.27	18.76	20.00	1.37	8.33	0.57	44.06	1574.80	106.61	3.02	107.87	7.30
50	32.70	9.22	22.62	13.33	0.79	4.44	0.26	40.76	144.58	58.93	2.42	8.57	3.49
60	40.00	3.80	35.83	13.33	0.75	3.70	0.21	33.33	350.79	37.20	1.88	19.74	2.09
70	48.85	19.28	38.25	26.67	2.19	6.35	0.52	54.60	138.33	69.73	4.48	11.36	5.73

effectiveness of 4.05. Effectiveness of chlorophyll mutations was maximum at 20 mM (22.23) and 30mM (0.70) EMS concentrations on M_1 plant and M_2 seedling bases respectivel:

The effectiveness of gamma rays in inducing viable mutations ranged from 19.04 (70kR) to 166.65 (20kR) and 1.64 (70kR) to 10.40 (20kR) on M_1 plant and M_2 plant bases respectively. The effectiveness of viable mutations was maximum at 20kR and minimum at 70kR both on M_1 plant and M_2 plant bases. Maximum effectiveness of 22.23 and 0.85 were obtained at 20mM EMS concentration on M_1 plant and M_2 plant bases.

Generally, effectiveness of chlorophyll and viable mutants were higher in gamma ray treatment than EMS both on M_1 plant and M_2 seedling bases. Rajasekaran (1973) in blackgram and Gunasekaran *et al.* (1998) in cowpea have reported that gamma rays were more effective than chemical mutagen in inducing viable mutations. In the present study, the effectiveness decreased with increase in concentration of EMS. This was in confirmation with the findings of Vanniarajan (1989) in blackgram, Jebaraj and Marappan (1981) and Packiaraj (1988) in cowpea.

Efficiency

Efficiency of chlorophyll mutations based on lethality, injury and sterility is presented in Table 3. On M_1 plant basis, gamma ray treatments were more efficient than EMS in producing chlorophyll mutations since maximum efficiency of 194.03 and 544.08 was obtained at 20kR based on lethality and sterility bases. But based on injury, EMS treatments were more efficient than gamma rays in producing chlorophyll mutants because at 40mM EMS concentration the efficiency recorded was 1574.80 on M_1 plant basis.

The efficiency of viable mutants on M_1 plant and M_2 plant bases is presented in Table 3. In gamma ray doses, on sterility basis maximum efficiency of 1360.41 and 84.70 was recorded at 20kR on M_1 plant and M_2 plant bases respectively. On lethality basis, maximum efficiency of 485.15 at 20kR on M_1 plant basis and 30.28 at 20kR on M_2 plant basis were obtained. In EMS concentration, based on injury maximum efficiency 1574.80 was obtained at 40mM on M_1 plant basis and 107.87 at 40mM on M_2 plant basis.

Generally it was inferred from the present study that 20kR gamma ray dose was more efficient on lethality and sterility bases and 40mM EMS concentration was more efficient on injury basis in inducing chlorophyll and viable mutations. Vanniarajan (1989) and Ahmed John (1995) have reported that gamma rays were more efficient than EMS in inducing chlorophyll and viable mutations in blackgram.

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