

EMS - Induced Mutagenesis in *Sorghum bicolor* (L.) Moench

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EMS effects on varieties IS 7155, BD 569 and the hybrid IS 7155 x BD 569 of *Sorghum bicolor* (L.) Moench were studied. Seedling characters, chlorophyll variants, cytological aberrations in M_1 , chlorophyll mutants and morphological mutants in M_2 were studied to assess the effects of EMS. Seedling characters decreased at 0.3% EMS in all the entries and however reduction was less in the hybrid indicating its resistance to the mutagen. The effectiveness of the mutagen to induce high frequency of chlorophyll variants, chlorophyll mutants, and other morphological mutants was observed. The effectiveness of the mutagen was dose dependent both in the parents and hybrid.

Key Words: Parents, Hybrid, Presoaking period, Chlorophyll variants; Mutants

Introduction

Ethyl methanesulfonate (EMS) is known to be mutagenic in a wide variety of organisms. EMS was found unambiguously to increase the proportion of mutants (Plaquet-type and host range) in T_2 phage treated *in vitro* with the chemical (Loveless 1958) and EMS has been used in mutational studies for a wide variety of biological test systems. The effects of EMS on some varieties of *Sorghum* was studied by several workers (Sharma 1965, Kapoor 1967, Sreeramulu 1970, 1971, 1974, Wanjari & Kutarekar 1977). Recently, Gary (1984) reviewed the genetic effects of EMS on plant and animal systems.

The effectiveness of a chemical mutagen depends not only on specific properties of tissues and organisms treated but also on the properties of the agent. The treatment conditions further influence the mutagenic action to a considerable extent. Presoaking seeds before mutagenic treatment increases synchronization of cells at division in embryonic tissue and caused by the partial lack of oxygen (Swaminathan 1969). It also renders the cell membranes more permeable to chemical mutagen, and thus a quicker and direct action is likely

to follow leading to alteration in mutation frequency. Konzak et al. (1964) observed that pre-soaking results in a higher efficiency of EMS action compared to dry seed treatment. There has been a considerable interest in the study of cytogenetic and related effects on *Sorghum* induced by physical and chemical mutagens. Such an approach provides a useful complementary method to the breeders in addition to the conventional techniques for developing elite varieties. In the present study an attempt has been made to investigate the mutagenic effectiveness of Ethyl methanesulfonate on parents and hybrid of *Sorghum bicolor* (L.) Moench with special reference to the concentration of the mutagen.

Materials and Methods

Dry well filled seeds of *Sorghum bicolor* varieties IS 7155 and BD 569 and their hybrid (IS 7155 x BD 569) with moisture content (14%) presoaked for 9 hrs were treated with 0.1, 0.2 and 0.3% EMS concentrations for 8 hrs, and later seeds were washed in running water for half an hour. For meiotic studies, immature spikelets were fixed in 1 : 4 acetic alcohol and smear prepara-

tions were made in 1% acetocarmine. Panicles of M_1 plants were harvested at random at maturity and sown in the seed beds to raise M_2 generation. The frequencies of chlorophyll deficient mutations per M_1 panicle and 100 M_2 seedlings were recorded.

Results and Discussion

In general, there was dose-dependent relationship in respect of germination, seedling height and leaf number. Only for survival there was no dose-dependent relationship between 0.1 and 0.2% EMS application. The response was particularly prominent at 0.3% EMS than at 0.1%. In general, the extent of reduction in germination, survival, seedling height and leaf number were more in the parents compared to the hybrid (table 1). This can be attributed to the homozygosity of the parent making it susceptible to the mutagen action and heterozygosity of the hybrid making it resistant to the

action of EMS in one way or other. Reduction in germination, survival and seedling height was of common occurrence in EMS-treated *Sorghum* (Sreeramulu 1970) and barley (Froese-Gertzen et al. 1964).

Chlorophyll variants appeared at all dose levels of EMS in both parents and hybrids. Striata category was the most frequent one, although xantha and zebra types were also noticed. The formation of stripes and their extent, position, colour and number showed high variation. The number of leaves carrying the stripes also varied ranging from one to all leaves in a plant. Special types of striped variants like green and yellow stripes intermixed, white leaves with green stripes and yellow leaves with green stripes were prominent. Heterozygosity of the hybrid lead to the heterotic effects in the production of more number of chlorophyll variants in M_1 and were proportional to the dose of mutagen applied. In general, EMS appeared to be efficient in the produc-

Table 1 Seedling parameters (% control)

Seedling characters	Control	IS 7155			BD 569			IS 7155 × BD 569		
		0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
Germination	100.00	95.97	93.95	70.46	77.69	85.38	63.07	114.66	111.33	80.00
Survial	100.00	100.00	103.67	66.91	95.41	96.33	66.05	115.27	120.13	82.63
Seedling height	100.00	93.72	90.45	55.96	85.50	80.76	68.19	107.63	104.71	93.54
Leaf number	100.00	94.62	89.57	87.05	89.39	86.36	82.72	95.16	87.91	86.17

Table 2 Frequency of cytologically aberrant plants in M_1 generation

Dose of EMS	IS 7155			BD 569			IS 7155 × BD 569		
	No. of plants screened	No. of aberrant plants	% aberrant plants	No. of plants screened	No. of aberrant plants	% aberrant plants	No. of plants screened	No. of aberrant plants	% aberrant plants
Control	5	—	—	5	—	—	5	—	—
0.1	35	4	11.42	30	4	13.33	42	2	4.76
0.2	36	8	22.22	37	6	16.89	39	3	7.69
0.3	38	2	5.26	40	6	15.00	32	8	25.00
Total	109	14	12.84	107	16	15.73	113	13	11.50

tion of chlorophyll variants in M_1 . Similar observations were also made earlier in *Sorghum* with different chemicals including EMS and also in combination with radiations (Kapoor 1967, Wanjari & Kutarekar 1977).

Out of 109, 107 and 113 plants screened in M_1 of IS 7155, BD 569 and IS 7155 × BD 569, 14 (12.84%), 16 (15.73%) and 13 (11.50%) respectively were found with meiotic aberrations (table 2). The frequency of aberrant plants was high in the parents, at 0.1 and 0.2% EMS doses, while in the hybrid a dose-dependent rela-

tionship of gradual increase of the aberrant plants with increase in the dose of EMS was evident. Less frequency of cytologically aberrant plants at 0.3 EMS in the parents can be attributed to the fact that the plants with deleterious cytological changes might have been eliminated at the seedling stage itself and hence less frequency of cytologically aberrant plants at 0.3 EMS in the parents and on the other hand heterozygosity of the hybrid made it more resistant at higher doses.

Cytological aberrations in M_1 recorded included

Table 3 Mature plant parameters of parents and hybrid in M_1 generation

Variety/Hybrid	Dose	Plant height (cm)	Leaf area (cm ²)	Panicle length (cm)	Panicle breadth (cm)	Seed set %	100 grain weight (g)
IS 7155	Control	207.91	437.26	21.80	3.53	67.64	1.59
		± 19.87		± 0.81	± 0.36	± 14.29	± 0.18
	0.1	192.59	397.40	19.36	3.14	37.89	1.29
		± 14.98		± 4.16	± 0.42	± 24.91	± 0.28
	0.2	149.23	320.16	17.21	3.27	7.52	1.16
± 23.76			± 3.80	± 0.65	± 4.91	± 0.19	
0.3	157.60	312.01	16.15	2.67	3.40	1.13	
	± 26.09		± 3.75	± 0.96	± 1.04	± 0.20	
BD 569	Control	112.00	402.15	29.55	4.70	59.55	2.50
		± 10.15		± 3.10	± 1.13	± 15.11	± 0.92
	0.1	100.00	400.11	26.21	3.95	40.15	1.26
		± 8.11		± 2.16	± 1.00	± 15.61	± 0.97
	0.2	102.12	397.21	24.00	3.99	20.21	1.09
± 11.12			± 1.11	± 1.91	± 21.55	± 0.18	
0.3	99.13	390.31	21.00	3.00	9.12	1.00	
	± 7.12		± 2.13	± 1.55	± 2.77	± 0.21	
IS 7155 × BD 569	Control	250.32	439.23	34.61	8.35	54.88	2.13
		± 42.67		± 1.01	± 1.79	± 30.72	± 0.91
	0.1	215.23	421.47	29.95	5.05	29.33	1.58
		± 42.27		± 3.36	± 1.18	± 19.39	± 0.32
	0.2	187.81	439.39	24.52	5.28	18.03	1.67
± 29.98			± 5.33	± 1.21	± 31.17	± 0.51	
0.3	186.29	391.19	24.63	4.25	3.11	1.49	
	± 44.43		± 5.55	± 1.19	± 1.77	± 0.31	

Table 4 Frequency of chlorophyll mutants in M_2 generation

Variety	Concentration of the mutagen (%)	M_1 Panicles			M_2 Seeding		
		No. analysed	No. mutated	%	No. analysed	NO. mutated	%
IS 7155	Control	10	—	—	218	—	—
	0.1	42	11	26.1	924	76	8.2
	0.2	16	2	12.5	133	2	1.5
	0.3	6	—	—	37	—	—
BD 569	Control	10	—	—	300	—	—
	0.1	21	7	39.3	1050	85	8.0
	0.2	20	3	15.0	850	45	5.2
	0.3	10	—	—	225	—	—
IS 7155 × BD 569	Control	10	—	—	257	—	—
	0.1	29	12	41.3	726	95	13.0
	0.2	15	—	—	78	—	—
	0.3	13	1	7.6	133	6	4.5

nucleolar budding at diakinesis, fragments at anaphase I and stickiness of chromosomes at metaphase I and anaphase I. Increase in rod bivalent formation and a low frequency of univalents lead to the reduction in chiasma frequency. Sreeramulu (1971) also observed decreased chiasma frequency in *Sorghum* due to mutagen treatment. Plant height decreased at all dose levels both in the parents and hybrid, with increase in dose of the mutagen. Leaf area, panicle length, panicle breadth, seed set %, and 100 grain weight decreased in both the parents and hybrid in treated populations. Seed set drastically decreased at 0.3 EMS dose level (table 3).

The frequency of chlorophyll mutants segregating in M_2 based on panicle as well as M_2 seedlings was more in the hybrid compared to the parents (table 4). The same situation prevailed in M_3 also. The reduction in frequency of chlorophyll mutations observed at higher doses of mutagen could be due to the increased size of the mutated sector (Gaul 1961) and consequently increased diplontic selection (Bekendam 1961, Yamaguchi 1962). The hybrid appears to be more sensitive than parents in inducing chlorophyll mutations and is evidently related to the heterozygosity.

Two desynaptic mutants were located in hybrid population of IS 7155 × BD 569 in M_2 generation. Variable number of univalents and increased number of rod bivalents were found at diakinesis and metaphase I. At anaphase I and II observed bridges appear to be due to the chromosomal stickiness.

Morphological mutants like dwarfs, plants with shrivelled endosperm, panicle non exersion type, loose panicle types and sterile plants were isolated in M_2 at lower frequencies (table 5). The production of dwarfs was more in the hybrid progeny. The extreme dwarf types isolated in the present study appear to be promising which can successfully used as a source of genetically controlled dwarfness.

In the present study, effect of EMS on variety IS 7155, BD 569 and the hybrid IS 7155 × BD 569 indicates that there is advantage of using hybrid systems to increase the variability and thus making the selection parameter more effective.

The production of chlorophyll mutants, dwarfs and desynaptics was more in the treated hybrid rather than the parents and at the same time the hybrid is somewhat resistant while the parents are mutagen-sensitive.

Table 5 Frequency of morphological mutants in M_2 generation

Variety Hybrid	EMS concentration	No. of plants screened	Mutant types					Total
			Dwarf mutant	Mutant with shrivelled endosperm	Non-exersion mutant	Loose pancile mutant	Sterile mutant	
IS 7155	Control	15	-	-	-	-	-	=
	0.1	75	1 (1.33)	-	-	-	2 (2.67)	3 (4.00)
	0.2	120	-	1 (0.83)	1 (0.83)	1 (0.83)	2 (1.67)	5 (4.16)
	0.3	90	-	-	-	-	3 (3.33)	3 (3.33)
BD 659	Control	75	-	-	-	-	-	-
	0.1	95	-	1 (1.05)	-	-	2 (2.10)	3 (3.15)
	0.2	91	-	-	2 (2.09)	-	-	2 (2.09)
	0.3	100	-	-	-	3 (3.00)	-	3 (3.00)
IS 7155 X BD 659	Control	75	-	-	-	-	-	-
	0.1	90	2 (2.22)	-	-	1 (1.11)	3 (3.33)	6 (6.66)
	0.2	95	1 (1.05)	-	-	-	-	1 (1.05)
	0.3	92	-	-	-	1 (1.08)	-	1 (1.08)

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