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1. An apparent interaction of chemical mutagens when applied in combination.

The alkylating agents, Diethylsulphate (DES), Ethylenimine (EI), Ethylmethanesulfonate (EMS), and Diepoxybutane (DEB), were applied in an aqueous solution to soaked and dry maize kernels singly and in all possible combinations. The homozygous genotype $\underline{A_1}$ $\underline{A_2}$ $\underline{Pr}$ $\underline{C}$ $\underline{Sh_1}$ $\underline{Wx}$ $\underline{Yg_2}$ was used in the endosperm marker technique. Pilot greenhouse and laboratory studies were conducted to establish a 70% survival rate for each chemical concentration and exposure.

The combination treatments were made by applying two single chemical treatments in succession, e.g. kernels were placed in the DES solution for 1 hour, rinsed in water and placed in the EI solution for 1 hour to produce the DES:EI treatment combination. The soaking pretreatment altered the response of the plant to individual chemicals, but not the overall maximum rate of mutation.

Tables 1 and 2 compare the effect of the pretreatments. Nearly all the treatments exceed the upper fiducial limit of the control for endosperm mutation rate. The combination treatments of EMS:DEB, DES:EMS, (Table 1), EMS:DES, and DEB:EI, (Table 2), produced a fourfold increase in mutation rate over that seen in the control and almost doubled the rate of the most effective single treatment. The agronomic data indicate no lethal response to successive treatments by two different chemicals, whereas a single treatment in one chemical concentration of similar duration will produce lethality. This synergistic response, which utilizes an apparent interaction of the chemical mutagens to produce a high rate of endosperm mutation without the usual loss of plant material, is a possible means of broadening the mutant spectrum and increasing the efficiency of chemical mutagens.

Table 1
Soaked Seed Pretreatment

Treatments			Plant Height	Pollen shed	Survival N = 45	Mutation rate	Fiducial limits .05 level
Chemical	Conc. M.	Time Hrs.	Ins.	*	%	%	
EMS:DEB			57	11	67	1.17	.9 — 1.5
DES:EMS			61	8	87	1.03	.8 — 1.4
EI:DEB			80	4	69	.95	.7 — 1.3
EI:DES			57	7	69	.80	.6 — 1.0
DEB:DES			61	3	78	.77	.6 — 1.0
EI	.050	1	70	9	53	.60	.4 — .9
DEB:EI			74	4	69	.55	.4 — .8
DEB:EMS			59	7	67	.52	.3 — .8
DES:DEB			70	8	56	.51	.4 — .7
DES	.045	1	67	3	93	.45	.3 — .6
EMS:EI			48	10	51	.43	.2 — .8
DEB	.003	1	89	1	67	.41	.3 — .6
DES:EI			62	10	53	.36	.2 — .6
EMS:DES			32	11	82	.33	.1 — 1.2
EMS	.010	12	61	6	84	.30	.2 — .4
EI:EMS			44	16	20	0	-- — --
Control			93	0	90	.36	.3 — .4

*Days after control.

Table 2
Non-soaked Seed Pretreatment.

Treatments			Plant Height	Pollen shed	Survival N = 45	Mutation rate	Fiducial limits .05 level
Chemical	Conc. M.	Time Hrs.	Ins.	*	%	%	
EMS:DES			48	9	80	1.16	.9 — 1.5
DEB:EI			80	6	7	1.00	.5 — 1.5
DES:EI			67	4	29	.79	.5 — 1.2
DES:EMS			58	8	95	.72	.3 — .9
EMS	.010	12	61	5	78	.65	.5 — .9
EI:EMS			61	10	33	.64	.4 — .9
EI:DEB			84	4	22	.59	.4 — .9
EMS:DEB			65	9	62	.55	.4 — .8
DEB:DES			81	1	98	.53	.4 — .7
DEB	.006	1	95	1	93	.52	.4 — .7
EI:DES			78	3	49	.47	.3 — .7
EMS:EI			61	7	56	.45	.3 — .9
DES:DEB			81	5	13	.38	.1 — .9

Table 2 Continued

Treatments			Plant Height	Pollen shed *	Survival N = 45 %	Mutation rate %	Fiducial limits .05 level
Chemical	Conc. M.	Time Hrs.	Ins.				
DES	.045	3	71	9	87	.31	.2 — .5
DEB:EMS			61	8	78	.29	.1 — .4
EI	.050	1	0	0	0	0	-- — --
Control			93	0	89	.27	.2 — .3

* Days after control.

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2. Location of small plant (spl) on chromosome 6.

Small plant (spl) mutant stocks were crossed to a series of stocks homozygous for waxy marked chromosome-nine translocations. The F_1 plants were selfed and F_2 starchy and waxy seeds from each translocation cross were planted separately and examined for small plant (spl) segregations.

Expected ratios (25%) of small plant were obtained with all translocations except T6-9$^{4505-4}$. Within the F_2 waxy seed class planted involving this cross a significant association was demonstrated between the small plant (spl) gene and the translocation tester T6-9$^{4505-4}$. Two hundred and seventy starchy and 230 waxy seeds from 7 selfed F_1 plants were planted. Not all of the starchy seeds were planted out for observation thus accounting for the discrepancy in the Wx:wx ratio. The data from the progenies involving T6-9$^{4505-4}$(6L.13 and 9 ctr.) were as follows: starchy seeds gave 158 normal:42 spl and 70 failed to grow; waxy seeds gave 148 normal:4 spl and 78 failed to grow. Progenies of waxy seed gave 2.6% small plants, from which it is apparent that small plant is located on chromosome 6 near the Y locus. However, there is a discrepancy in the progenies of the starchy seed since fewer small plants were observed than expected. Testcrosses have been made and will be analyzed to confirm the location and linkage on chromosome 6.

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