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1. New endosperm mutant tentatively designated opaque-4.

An opaque phenotype endosperm mutant has been isolated from an "exotic" composite. Negative allele tests have been obtained with du, h, bt_1, bt_4, o_1, o_2 and ae. Also, it does not show the floury phenotype dosage effect. Analyses show that it is normal in amylose level and in lysine content.

Paul L. Crane

2. Induced mutation rates produced by treatments with four alkylating agents to the proembryo of Zea mays L.

The study reported here involves the use of seedling marker genes Lg_1 and Gl_2 at positions 11 and 30 in the short arm of chromosome 2 and Yg_2 at position 7 in the short arm of chromosome 9 as a system of testing and comparing the mutagenicity of ethyl methanesulfonate (EMS), diethyl sulfate (DES), ethylenimine (EI), and diepoxybutane (DEB) treatments applied to the proembroyos of maize.

Homozygous lg_1 gl_2, Yg_2 C Sh_1 Bz Wx female stocks were crossed with homozygous Lg_1 Gl_2, yg_2 c sh_1 bz wx male stocks. The proembryos 24 and 48 hours after pollination were treated with 20 ml solutions of one of the four alkylating agents. The treatment concentrations for each of the agents were as follows: EMS-0.2, 0.1, and 0.01653M; DES-.045M; EI-0.2, 0.1, and 0.05M; and DEB-0.01, 0.005, and 0.0025M. As a control, deionized glass-distilled water was used. All solutions were freshly prepared in deionized glass-distilled H_2O at pH 6.4 with phosphate buffer. The proembryos were prepared by carefully making a longitudinal incision in the ear shoot, plying back the husks from the ear sufficiently to allow one to wrap absorbent cotton around the ear. The ear shoots were soaked with the treatment solutions and covered with a bag. The cotton swab was allowed to remain for 2 hours and then it was removed. The ear was thoroughly washed with deionized glass-distilled H_2O, the husks were closed back around the ear and held by rubber bands and the ear was covered with a bag.

The mature ears were scored for genetic losses of partial and whole endosperm and seedling markers and are shown in table 1. This communication reports only the results of the pooled genetic losses of seedling markers. The mutant phenotypes were scored in seedling material from the first through the sixth leaf stage. Many seedling mutation events were also scored as very minute streaks of recessive tissure in addition to those partial events which were 1/2, 1/4, 1/8, 1/16th part of the seedling leaf.

Table 1

Pooled seedling mutation rates following treatment of maize zygotes and proembryos 24 and 48 hours after pollination by EMS, DES, EI and DEB.

Treatment No.	Chemical conc. (M)	Age of zygote or proembryo at treatment (hrs.)	No. of seedlings	Total mutation rate %	Limits[a] .05 level
1	EMS 0.2	24	380	28*	23.27-32.37
2	EMS 0.2	48	72	28*	18.06-39.62
3	EMS 0.1	24	1100	25*	22.23-27.37
4	EMS 0.1	48	756	13*	10.47-15.56
5	EMS 0.01653	24	1685	5*	3.57- 5.62
6	EMS 0.01653	48	561	7*	6.07- 8.98
7	DEB 0.01	24	220	10*	6.40-14.75
8	DEB 0.01	48	77	6	2.14-14.56
9	DEB 0.005	24	246	7*	4.40-11.00
10	DEB 0.005	48	191	5	2.54- 9.41
11	DEB 0.0025	24	450	5*	3.46- 7.84
12	DEB 0.0025	48	167	5	2.49-10.00
13	D-H_2O --	24	2738	3	2.13- 3.38
14	D-H_2O --	48	697	4	2.46- 5.42
15	DES 0.045	24	691	10*	7.48-11.99
16	DES 0.045	48	275	9*	5.69-12.70
17	EI 0.2	24	588	6*	4.19- 8.19
18	EI 0.2	48	136	10	5.74-16.68
19	EI 0.1	24	861	2	1.52- 3.71
20	EI 0.1	48	217	5	2.56- 8.88
21	EI 0.05	24	315	0	0- 1.17
22	EI 0.05	48	50	0	0- 7.38

a/ Calculated according to Stevens, 1942.

*/ Mutation rate exceeds respective aged control at .05 level of significance.

Ethyl methanesulfonate produced the greatest seedling mutation yield (28%). The 0.2M EMS solutions applied to both 24 and 48 hour old proembryos yielded 7 to 9 times their respective D-H_2O controls and about 3 times the treatments which gave the highest rate of loss of genetic markers in each of the DES, EI, and DEB chemical treatments. The latter treatments were 2 to 3 times greater in mutation yield than their respective controls. All of the EMS and DES treatments were significantly better than control when applied to both 24 and 48 hour old proembryos. However, the DEB solutions at all concentrations used were only significantly greater than control when applied to the 24 hour old proembryos. The 0.2M solution was the only EI treatment which was significantly greater than control. With the exception of the 0.1M EMS treatments, there were no significant differences in mutation yield between the 24 and 48 hour old proembryos treated with the alkylating agents at each of the treatment concentrations. For each of the chemical treatments where there was a concentration gradient, in general there was an increase in the rate of loss of genetic markers with an increase in concentration.

It was particularly noted that EMS produced a high proportion of 1/2 to 1/16th part leaf sectors showing the genetic loss for $\underline{Yg}_2$ and $\underline{Gl}_2$ in addition to the small streaks. The larger sectors produced in proembryo treatments encourage the use of this type of treatment for screening for true gene mutations since the chance of survival against diploidal elimination of mutant sectors seems greater. The induction of several single locus whole seedling mutations by EMS for either $\underline{Yg}_2$ or $\underline{Gl}_2$ and 3 multiple locus whole seedling mutants for both $\underline{Yg}_2$ and $\underline{Gl}_2$ strengthens the suggestion that perhaps EMS is producing "true" gene mutations at the substructural level of the chromosome.

D. V. Glover

3. Further tests for the location of small plant (spl) on chromosome 6.

The location of a small plant (spl) character has been shown to be on chromosome 6 near the Y locus (MGCNL 39:152, 1965). Further evidence that it is on chromosome 6 comes from the following testcross data in the presence of a series of waxy and chromosome-nine translocations.

Small plant (spl) mutant stocks were crossed to stocks homozygous for the waxy marked chromosome-nine translocations. The F_1 plants were backcrossed to a small plant waxy line. The starchy and waxy seeds from each translocation cross were planted out separately and the plants were classified for small plant (spl) segregations. A chi-square test for independence, utilizing fourfold contingency tables with one degree of freedom, was used to determine if the populations from the two classes of seeds