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1. Induction of endosperm mutations in maize with ethyl methanesulfonate.

Methods for evaluating the relative genetic effect of mutagenic treatments and for obtaining endosperm mutations throughout the entire genome of an agronomically desirable line of maize are needed to facilitate fundamental and applied mutation research in maize. One method commonly used by maize workers is to treat a dominant stock, cross it to a genetic tester stock that is recessive for several known loci, and score for recessive mutants in the M_1 generation. However, this method is not well suited for identifying and accumulating endosperm mutations occurring elsewhere in unmarked regions throughout the chromosomes.

Most of the mutations used by geneticists and plant breeders have arisen spontaneously, hence at a low frequency. Therefore, the chemical mutagen ethyl methanesulfonate (EMS) was used because its ability to induce a high mutation frequency has been well established. The use of this mutagen with an efficient screening procedure can show the relative genetic response to mutagenic treatments; in addition, mutations potentially useful to geneticists and plant breeders may be obtained.

Inducing endosperm mutations directly into an agronomically desirable line of maize may have three possible uses to plant breeders: (1) Mutants may be produced that are not known to have arisen spontaneously and their usefulness can be determined. (2) The amount of a specific desirable product may be increased by increasing the number of genes that govern its production. (3) Mutation may be more expedient than backcrossing to incorporate genes into desired lines. Endosperm mutations can also be useful to geneticists as genetic markers and in studies on biochemical pathways.

Mutants in many self-pollinated plants, e.g., barley, are readily recovered by growing seeds of individual heads of the M_1 plants, since the male and female organs are in the same flower. Even if the mutant

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involves only a small sector, most segregate in the M_2 generation. In maize, however, small mutant sectors may not involve both the ear (female inflorescence) and tassel (male inflorescence) and will not segregate in the M_2 generation. A mutation in either the ear or tassel would result in a heterozygous plant in the second generation which segregates in the third generation. Singleton and co-workers, who have done research on this problem, state that this difficulty could be overcome by using the one-celled proembryo as the experimental material, which affords an opportunity of obtaining a nonchimeric plant (Chatterjee et al., Genetics 52:1101-1111, 1965). Singleton (Genetics 52:475, 1965) has further pointed out that mutagenic treatments on seeds could be used more effectively if the treated seeds were planted in an isolated field and allowed to interpollinate for one generation before an attempt was made to recover the mutants. In this manner mutants could be readily recovered if a generation of random mating (M_1) were followed by self-pollination of the M_2 generation. Every mutant would be in the heterozygous condition and involve a whole plant, not a sector. The purpose of carrying mutagen treated material into subsequent generations in this research was to provide plant geneticists and breeders an opportunity to develop plant and endosperm mutations in the entire genome of maize for use in their research as well as to obtain an estimate of the effects of mutagenic treatments.

Seeds of the inbred line M14 were treated with 0.005 or 0.01 M EMS (Eastman Organic Co.) for 10 h at 25° ± 0.02° C in a 0.02 M aqueous phosphate buffer (pH 7.5). The seeds had been stored at 60% relative humidity and had a moisture content of approximately 11.5%. After treatment they were rinsed in distilled water and sown immediately in the field. The material was planted in isolation, and the M_1 plants were self-pollinated. Forty-five M_1 ears were obtained from the 0.005 M treatment and 55 M_1 ears from the 0.01 M treatment. In the next generation, 30 seeds were planted from each M_1 ear, and the plants were later thinned to 15 per row. These plants were self-pollinated, and M_2 ears with M_3 seed on them were harvested and visually scanned for mutants segregating on the ears.

The procedure used in handling the treated material through subsequent generations was as follows:

M_1 seed	M_2 seed
↓	↓
M_1 plant (ears)	M_2 plant (ears)
↓ self-pollinate	↓ self-pollinate
M_2 seed on ear	M_3 seed on ear
FIRST POLLINATION	SECOND POLLINATION

An M_1 seed (normal or wild-type seed treated with a mutagen) is planted; this seed produces an M_1 plant, which is self-pollinated and has M_1 ears with M_2 seed on them. These M_2 seeds are planted in a subsequent growing season and in turn produce M_2 plants, which are self-pollinated and have M_2 ears with M_3 seed on them. Mutants produced by treating the M_1 seed with a mutagen will segregate in the M_3 seed generation. Since the M_1 seed has tissue made up of mutated and nonmutated cells, this procedure of self-pollinating the plants twice essentially isolates the progeny of a single mutated cell.

The mutants were detected in the M_3 seed generation, but the material was pedigreed from an M_1 ear. Therefore, the number of independently occurring mutations was divided by the number of M_1 ears to give the M_1 mutation frequency. To obtain the M_3 mutation frequency the number of independently occurring mutations was divided by the number of ears with M_3 seed on them.

Sterility occurring on ears bearing M_3 seed could be separated into two classes, i.e., ¼ or ½ sterility. When sterility occurred with the mutants (Tables 1 and 2), its percentage was calculated by a method similar to that used for obtaining the mutation frequency. That is, to calculate sterility on an M_1 ear basis for a particular phenotypic class the number of M_1 ears was divided into the number of mutant ears that had sterility in that phenotypic class. The M_3 sterility frequency was calculated in a like manner, with the number of M_3 ears being the divisor. If an ear is ¼ sterile the reason for it is assumed to be genetic; if the ear is ½ sterile the reason is assumed to be chromosomal, i.e., a chromosomal rearrangement. If several ears in a progeny row (ears with M_3 seed) were

Table 1

Phenotypes and mutation and sterility percentages from an ethyl methanesulfonate treatment (0.005 M, seeds planted wet)

Phenotype	All mutants			Mutants grown			Mutants that produced plants			Mutants that produced seed		
	M_1	M_3	Nr.	M_1	M_3	Nr.	M_1	M_3	Nr.	M_1	M_3	Nr.
Slightly opaque	11.11	0.99	5	6.67	0.59	3	6.67	0.59	3	6.67	0.59	3
Sterile*	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1
Translucent, tarnished												
Translucent, shrunken	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1
Wrinkled, glassy												
Opaque												
Floury	13.33	1.18	6	6.67	0.59	3	4.44	0.39	2	4.44	0.39	2
Lemon												
Orange												
White												
Miscellaneous												
Total	26.66	2.37	12	15.56	1.38	7	13.33	1.18	6	13.33	1.18	6
Sterile*	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1
45 M_1 ears												
507 M_3 ears												

*¼ sterility

segregating for sterility, it was assumed to be due to a single gene or to one chromosomal rearrangement and was used in this way to calculate the M_1 and M_3 sterility percentages. However, the sterility could be from separate gene mutations or chromosomal rearrangements.

Barley researchers use a system to classify the different chlorophyll deficient mutants. To make this maize mutation research more meaningful a classification system for the mutants was devised. The phenotypes used in this classification system, as well as mutation and sterility percentages from two EMS treatments, are given in Tables 1 and 2. The phenotypes of the mutants are based on the classification system as reported by Kramer et al. (Agron. J. 50:207-210, 1958) and by Creech (Genetics 52:1175-1186, 1965). However, additional phenotypic classifications were needed for this research. The phenotypic classification (Tables 1 and 2) does not imply genotype or allelism. Therefore, one must be cognizant of the definite distinction between the phenotypic classification in these tables and the genotypic classification of existing genes that have the same names, e.g., as used by Kramer and in this paper the opaque phenotype is the waxy genotype, and there are genotypes that are opaque (as opaque-1 and opaque-2). The classification system has enough different phenotypes to include the mutants that have occurred in the various experiments to date, even though in these two experiments mutants were not observed for each of the phenotypic classes; also additional phenotypes could easily be added to the classification system. The miscellaneous class was used for rarely occurring mutants or ones that were combinations of the other phenotypes.

The number of mutations and mutation frequencies by phenotypic classification is given for both the M_1 and M_3 generations (Tables 1 and 2). The mutants are classed as "all mutants," i.e., all of those detected. The classification "mutants grown" included those actually selected for planting in the field. The classifications "mutants that produced plants" and "mutants that produced seeds" are self explanatory. The total mutation frequency for an experiment and the total number of M_1 ears and the number of ears that had M_3 seed on them are given at the bottom of the tables.

Table 2

Phenotypes and mutation and sterility percentages from an ethyl methanesulfonate treatment (0.01 M, seeds planted wet)

Phenotype	All mutants			Mutants grown			Mutants that produced plants			Mutants that produced seed		
	M_1	M_3	Nr.	M_1	M_3	Nr.	M_1	M_3	Nr.	M_1	M_3	Nr.
Slightly opaque	32.73	3.42	18	9.09	0.95	5	3.64	0.38	2	3.64	0.38	2
Sterile*	3.64	0.38	2	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0
Sterile**	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1
Translucent, tarnished	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1
Translucent, shrunken	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1
Wrinkled, glassy	1.82	0.19	1	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0
Sterile*	1.82	0.19	1	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0
Opaque	3.64	0.38	2	3.64	0.38	2	3.64	0.38	2	1.82	0.19	1
Floury	10.91	1.14	6	7.27	0.76	4	3.64	0.38	2	0.00	0.00	0
Sterile**	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Lemon	7.27	0.76	4	7.27	0.76	4	5.45	0.57	3	3.64	0.38	2
Sterile*	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1
Orange												
White	1.82	0.19	1	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0
Miscellaneous	5.45	0.57	3	3.64	0.38	2	0.00	0.00	0	0.00	0.00	0
Sterile*	3.64	0.38	2	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0
Total	67.28	7.03	37	38.19	3.99	21	20.01	2.09	11	12.74	1.33	7
Sterile*	10.92	1.14	6	7.28	0.76	4	1.82	0.19	1	1.82	0.19	1
Sterile**	3.64	0.38	2	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1
55 M_1 ears; 527 M_3 ears												

*¼ sterility

**½ sterility

The M_1 mutation frequency for the 0.005 M EMS treatment is 26.66%, and at this dose level not all of the phenotypic classes have mutants in them. However, a dose of 0.01 M EMS produced an M_1 mutation frequency of 67.28% and gave a wider spectrum of mutations, i.e., mutants occurred in all but one phenotypic category. Also, the sterility percentage was increased with a 0.01 M EMS treatment compared with 0.005 M.

After the mutants were observed on the ears, the usual mutation frequencies were calculated ("all mutants"). Some mutants ("mutants grown") were selected to be grown to the M_4 generation, since one objective of this experiment was to determine whether viable and useful mutants could be obtained. The main reason not all mutants were grown to the M_4 generation was that some, in addition to being endosperm mutants as listed in the tables, had seeds that were miniature.

If sterility occurred in a particular phenotypic classification, the type (¼ or ½ sterility) is designated and the total sterility of the mutants in the experiment is given at the bottom of the table. For example, in Table 1 five mutants were classified as slightly opaque, and one of the five mutant ears was ¼ sterile.

A disadvantage of EMS has been that sterility is produced in the progeny of treated material. However, this research indicates that it is possible to obtain mutants that do not have sterility (Tables 1 and 2).

The usual nursery procedure is to overplant and then thin to 15 plants per nursery row; however, to conserve seed only 15 seeds of each mutant were planted, and in many cases the survival in a row was less than this number. Also, some of the mutants (entire rows of M_3 seed), did not survive in the field. With the 0.005 M EMS treatment 86% of the mutants planted grew and produced seed. With the 0.01 M treatment 52% of the mutants grew and 33% produced seed.

Mutations can be obtained after one self-pollination in maize if the same mutated cell goes to make up both the tassel and the ear. In only one instance in these two experiments were all the kernels on the ear (M_3 seed) of the mutant class, i.e., the mutation was present after the first pollination but was not detected. As proposed by Singleton and shown by this research, the material should be carried to the M_3 generation to obtain any appreciable number of mutations.

The type of induced mutations (point mutations or deletions) at the waxy locus has been studied at this laboratory. The method reported here should produce homoallelic waxy mutants and in the same genetic background as that to which the standard waxy alleles have been backcrossed. Singleton has proposed allowing the M_1 plants to open pollinate (rather than selfing them as we did) and then selfing them in the next generation, at which time the heterozygous plants will segregate. If the material were allowed to open pollinate, a single mutation in the tassel could be disseminated in the field and be detected several times, but in fact it would be only one mutation. Also, we were interested in calculating the M_1 and M_3 mutation frequencies, and to do this we pedigreed the material by keeping the progeny from a particular M_1 plant. The second generation has to be self-pollinated. We self-pollinated both generations for the above reasons and because in our nursery procedure the number of M_1 plants is less by approximately a factor of 15 than the number of plants in the second generation.

When the endosperm mutants (M_3 seed) were planted, some of them segregated for chlorophyll deficient or dwarf plants; this indicates that probably a gene for chlorophyll deficiency or dwarfism mutated and was segregating independently from the endosperm mutation. Some of the endosperm mutants produced all chlorophyll deficient or dwarf plants--this may be an example of pleiotropism. Among the chlorophyll deficient seedlings, a case was observed which may represent the lemon-white (lw) series of genes, i.e., lemon endosperm-white seedling.

It should therefore be possible to obtain plant mutations at frequencies dependent on the dose of EMS if the M_3 seeds were planted, even if endosperm mutants had not been detected on the ears. For example, chlorophyll deficient and morphological mutants should be observed. Some of these mutants may be useful to investigators also.

For mutagen treatments to be used to introduce a particular mutant into a line and hence eliminate backcrossing, it is vital to have no genetic or chromosomal sterility concomitant with a mutant; also, one mutant per plant is desired, which may be attainable with a proper dose of EMS. After an induced mutant is obtained in a line, it may be necessary to backcross it to the original line to eliminate sterility or a

second mutant gene. However, fewer backcrosses would be required than if an unrelated non-recurrent parent were used with conventional backcross procedures.

Apparently EMS can be used to produce cytoplasmic mutants in plants (Dulieu, Mutation Res. 4:177-189, 1967) and may be useful to produce cytoplasmic sterility in maize by the above outlined procedures; probably more important, cytoplasmic sterility may be produced in other species with some modification of these procedures.

Robert W. Briggs

2. Modification of the efficiency and effectiveness of ethyl methanesulfonate treatments in maize.

The action of ethyl methanesulfonate (EMS) on seeds was investigated by altering post-treatment conditions so as to modify the genetic effects and physiological damage produced. Genetic effects were measured by the frequency of yellow green (yg_2) sectors in the leaves of Yg_2/yg_2 seedlings; physiological damage by reduction in plant height. If seeds are dried immediately after treating with 0.01 M EMS (10 h, 25° C), the height of seedlings will be reduced significantly. If, however, the seeds are soaked for 4 days (at 3° C) after EMS treatment, then dried, the physiological damage is minimized and equals the control value. This post-treatment condition also reduces mutation rate, but it maximizes the treatment efficiency (ratio of yg_2 frequency to plant height reduction). Post-soaking apparently removes EMS and its hydrolysis products which are particularly harmful when the seeds are dried. However, post-soaking EMS-treated seeds before drying reduced the effectiveness of the treatment, as measured by the ratio of yg_2 frequency to dose of mutagen. This is probably because unhydrolyzed (active) EMS was removed from the seeds.

Robert W. Briggs

3. Esterase isozymes: new loci.

The E_1 through E_4 esterases were described by Drew Schwartz and his students at Indiana. The present note briefly describes several new anodal esterase loci and a single transaminase locus found during an ongoing classification of enzyme polymorphisms in flowering plants carried out at the University of Hawaii.