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1. Further studies on induction of endosperm mutations in maize with ethyl methanesulfonate.

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.

Procedures to induce endosperm mutations in an inbred line of maize with EMS and the utilization of these mutations have been previously reported (2). In the first experiments the mutagen treated seeds were planted wet; in the studies reported here the treated seeds were dried before planting. Advantages of drying mutagen treated seeds compared to planting them wet were given by Briggs (1).

Mutation research in maize needs to be conducted differently than in self-pollinated plants. 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 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 the mutant will not segregate in the M_2 generation. A mutation in either the

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ear or tassel would result in a heterozygous plant in the second generation which segregates in the third generation. These differences were pointed out by Singleton (5) when he proposed procedures of inducing mutants in maize. Briggs (2) confirmed his procedures and further data were reported by Singleton (6).

Singleton 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 0.05 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 soaked at 3° ± 0.02° C in distilled-deionized water for 48 hrs. The contents of the treatment flasks, in which the water was changed every 24 hrs., were agitated by a platform shaker operated to make 75 excursions per min. After post-soaking, the seeds were dried for 72 hrs. in a room maintained at 22° C and 60% relative humidity. The material was planted in isolation, and the M_1 plants were self-pollinated. Sixty-eight M_1 ears were obtained from the 0.005 M treatment and 77 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.

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.

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. Further details on calculating sterility can be found in last year's Maize News Letter (2). If an ear is ¼ sterile the cause of sterility is assumed to be genetic; if the ear is ½ sterile the cause is assumed to be chromosomal, i.e., a chromosomal rearrangement.

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. (4) and by Creech (3). However, additional phenotypic classifications were needed for this research. The phenotypic classification (Tables 1 and

Table 1

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

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	2.94	0.25	2	2.94	0.25	2	1.47	0.12	1	1.47	0.12	1
Translucent, tarnished	4.41	0.37	3	2.94	0.25	2	1.47	0.12	1	1.47	0.12	1
Sterile*	1.47	0.12	1	1.47	0.12	1	1.47	0.12	1	1.47	0.12	1
Translucent, shrunken	1.47	0.12	1	1.47	0.12	1	1.47	0.12	1	1.47	0.12	1
Wrinkled, glassy	—	—	-	—	—	-	—	—	-	—	—	-
Opaque	1.47	0.12	1	1.47	0.12	1	1.47	0.12	1	1.47	0.12	1
Floury	29.41	2.46	20	13.24	1.11	9	11.76	0.98	8	5.88	0.49	4
Sterile*	1.47	0.12	1	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Lemon	8.82	0.74	6	8.82	0.74	6	5.88	0.49	4	4.41	0.37	3
Orange	—	—	-	—	—	-	—	—	-	—	—	-
White	—	—	-	—	—	-	—	—	-	—	—	-
Miscellaneous	5.88	0.49	4	4.41	0.37	3	4.41	0.37	3	1.47	0.12	1
Sterile*	1.47	0.12	1	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Total	54.40	4.55	37	35.29	2.96	24	27.94	2.34	19	17.65	1.48	12
Sterile*	4.41	0.36	3	1.47	0.12	1	1.47	0.12	1	1.47	0.12	1
68 M_1 ears												
813 M_3 ears												

*¼ sterility

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 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.

The M_1 mutation frequency for the 0.005 M EMS treatment was 54.40% and the sterility was 4.41%. However, the higher dose of 0.01 M produced a mutation rate of 36.37% and the sterility was 13.00%. This may have been because a 0.01 M EMS treatment produced a relatively large amount of plant damage in the first generation; hence many cells were killed that may have had mutations. The fact that the 0.01 M treatment produced more sterility than the 0.005 M treatment was probably a reflection of this damage from the higher dose. This relationship of relatively high sterility of the 0.01 M treatment compared to the 0.005 M treatment exists in the M_4 seed generation.

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. With the 0.005 M EMS treatment 79% of the mutants planted grew and 50% produced seed. With the 0.01 M

Table 2

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

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	2.60	0.35	2	2.60	0.35	2	2.60	0.35	2	1.30	0.17	1
Translucent, tarnished	9.09	1.21	7	9.09	1.21	7	5.19	0.69	4	3.90	0.52	3
Sterile*	3.90	0.52	3	3.90	0.52	3	2.60	0.35	2	2.60	0.35	2
Translucent, shrunken	2.60	0.35	2	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Sterile*	1.30	0.17	1	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Wrinkled, glassy	—	—	–	—	—	–	—	—	–	—	—	–
Opaque	—	—	–	—	—	–	—	—	–	—	—	–
Floury	5.19	0.69	4	3.90	0.52	3	1.30	0.17	1	1.30	0.17	1
Sterile*	1.30	0.17	1	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Lemon	9.09	1.21	7	7.79	1.04	6	5.19	0.69	4	5.19	0.69	4
Sterile*	3.90	0.52	3	2.60	0.35	2	1.30	0.17	1	1.30	0.17	1
Orange	3.90	0.52	3	3.90	0.52	3	3.90	0.52	3	3.90	0.52	3
Sterile*	1.30	0.17	1	1.30	0.17	1	1.30	0.17	1	1.30	0.17	1
White	—	—	–	—	—	–	—	—	–	—	—	–
Miscellaneous	3.90	0.52	3	2.60	0.35	2	1.30	0.17	1	0.00	0.00	0
Sterile*	1.30	0.17	1	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Total	36.37	4.85	28	29.88	3.99	23	19.48	2.59	15	15.59	2.07	12
Sterile*	13.00	1.72	10	7.80	1.04	6	5.20	0.69	4	5.20	0.69	4
77 M_1 ears; 578 M_3 ears												

*¼ sterility

treatment 65% of the mutants grew and 52% produced seed.

More discussion on utilization of EMS treatments in maize and its application to plant breeding can be found in last year's Maize News Letter (2).

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(3) Creech, Roy G. (1965) Genetics 52:1175-1186.

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1. Dominant ramosa ear character.

A few years ago Ing. Ramón Covarrubias Celis found plants with ramosa ears in the variety Yucatán 85 of the race Nal-Tel while he was a professor of genetics at the Graduate School of Chapingo, Mexico. His preliminary observations showed that this character was a dominant one not previously reported and probably controlled by a single gene.

Ramosa eared plants were crossed with normal plants from Yucatán 7 (Race Nal-Tel) and V520C (Race Tuxpeño). All the F_1 plants had the ramosa ear character showing that the trait is dominant over the normal. By sib pollinations the F_2 was obtained and normal and ramosa plants in the F_2 generation were counted. The results are given in Table 1.