

### 3. The effects of X-radiation on maize pollen.

Before crossing, pollen grains of the male parent in a cross of Wilbur's Flint X tester, having the genotype  $A_1 C R pr su$ , were subjected to acute X-radiation with a dose of 1500r. Among the  $F_1$  individuals, one of the plants which was shown to contain aberrations was self pollinated. All of the 51 seeds from this plant were grown last summer. Forty-one of them germinated and grew into mature plants. Twenty-three plants were available for chromosome studies. The inflorescences were collected and fixed and the anthers were squashed by following standard acetocarmine squash techniques. Of the 23 plants studied, one was found to contain a fragment. This fragment was measured at pachytene and found to be approximately 29.5u. Another plant frequently showed a bridge at anaphase I, but no fragment accompanying the bridge was observed. A further study is in progress to trace the fragment through to the quartet stage.

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### 1. Studies on induction of cytoplasmic male sterility with ethyl methanesulfonate.

"Apparently EMS can be used to produce cytoplasmic mutants in plants . . . and may be useful to produce cytoplasmic sterility in maize . . . probably more important, cytoplasmic sterility may be produced in other species . . ." (1). This statement was made based on research started at Brookhaven National Laboratory in 1967. At that

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\*Portions of this research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

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time the induction of cytoplasmic male sterility in maize was more academic than pragmatic. Following the discovery that corn with Texas male sterile cytoplasm is more susceptible to yellow leaf blight (Phyllosticta sp) and southern leaf blight (Helminthosporium maydis, Race T) than normal cytoplasm, research on detection of a new source of cytoplasmic male sterility is timely. Research on using EMS to induce cytoplasmic male sterility was started at Brookhaven National Laboratory during 1967 and has continued here at Funk Bros. Seed Co.

Initially, the inbred M14 was used and since 1969 W22 was used in these studies. To date, 18,087 plants have been examined to detect cytoplasmic male sterility, including treated and control populations. The total was made up of M14 treated with various doses of EMS, 3,045; W22 treated with various doses of EMS, 8,746; W22 control, 6,296 (Table 1).

In the first generation after EMS treatment, the plants were self pollinated. In the next generation, the self pollinated material was planted ear-to-row. Sterile plants in these progeny rows were crossed with the untreated inbred parent. Out of 818 of these progeny rows, 49 had from 1 to 5 male sterile plants. The male sterile plants, after being crossed with the untreated inbred parent, were grown in a subsequent generation. If any of this material was sterile, it was again crossed with the untreated inbred parent; this happened in three cases. One of the cases (2 ears) will be checked in the next generation to determine if it will remain male sterile. This is in a control population.

Material that was sterile after the first self pollination could be sterile due to a recessive or dominant gene or cytoplasmic factor. Plants remaining sterile in the next generation could be due to a dominant gene or cytoplasmic sterility factor. Plants sterile and not segregating in the next generation would be due to cytoplasmic factors. Therefore, to date no cytoplasmic sterile plants were induced by EMS and one control population remains to be checked in the next generation for the presence of cytoplasmic male sterility. Treatment procedures and further references to this work can be found in previous Maize Newsletters (1, 2).

Table 1  
Ethyl methanesulfonate treatments (10 hours at 25° C.)  
and progenies with sterile plants

| Inbred | Mutagen Treatment | Nr. M <sub>1</sub> Rows | Nr. M <sub>2</sub> Plants | Nr. M <sub>1</sub> Rows With Steriles |
|--------|-------------------|-------------------------|---------------------------|---------------------------------------|
| M14    | 1                 | 45                      | 675                       | 0                                     |
| M14    | 2                 | 63                      | 945                       | 3                                     |
| M14    | 3                 | 64                      | 960                       | 1                                     |
| M14    | 4                 | 26                      | 390                       | 1                                     |
| M14    | 5                 | 5                       | 75                        | 0                                     |
| W22    | 3                 | 308                     | 8,009                     | 22                                    |
| W22    | 4                 | 47                      | 737                       | 6*                                    |
| W22    | Control           | 260                     | 6,296                     | 16**                                  |
|        |                   | 818                     | 18,087                    | 49                                    |

1 - 0.005M 10 hrs. @ 25° C., planted wet.

2 - 0.01M 10 hrs. @ 25° C., planted wet.

3 - 0.005M 10 hrs. @ 25° C., post-soaked 3° C. 48 hrs., dried 72 hrs. at 60% R.H.

4 - 0.0075M 10 hrs. @ 25° C., post-soaked 3° C. 48 hrs., dried 72 hrs. at 60% R.H.

5 - 0.01 M 10 hrs. @ 25° C., post-soaked 3° C. 48 hrs., dried 72 hrs. at 60% R.H.

\* - 2 with sterile plants in next generation but fertile in following generation.

\*\* - 1 with sterile plants in next generation, remains to be checked in following generation.

EMS has been amply demonstrated to be a good mutagen of nuclear genes. Hence it might be a good cytoplasmic mutagen if the genetic material is similar for both methods of inheritance. There are a few reports of the induction of cytoplasmic mutants in plants. Dulieu (3) used EMS to induce chlorophyll deficient mutations in Nicotiana that were maternally inherited. Favret and Ryan (4) have induced cytoplasmic male sterile mutants in barley with x-rays and with EMS. Also, Lysikov et al. (5) reported that cytoplasmic male sterility has been induced in maize by chemical and physical mutagens.

Failure to detect cytoplasmic male sterility in the EMS treated material may be due to relatively small populations used; also, cytoplasmic male sterility may occur at a very low frequency.

#### References

- (1) Briggs, Robert W. (1969) Maize Genetics Newsletter 43:23-31.
- (2) Briggs, Robert W. (1970) Maize Genetics Newsletter 44:11-17.
- (3) Dulieu, H. (1967) Mutation Research 4:177-189 (In French).
- (4) Favret, E. A. and G. S. Ryan (1966) Mutations in Plant Breeding, pp. 49-61. International Atomic Energy Commission, Vienna.
- (5) Lysikov, V. N., A. N. Konotop, O. V. Eljandor and S. G. Byrka. (1970) Plant Breeding Abstracts. Vol. 40.

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#### 1. The effect of red and far-red light interruptions on paramutant R expression.

In MGCNL Vol. 43 we reported that the level of aleurone pigment produced by R' (paramutated R) was directly related to the number of dark periods administered to seedlings at an early stage of tassel initiation. We have been concerned with defining more closely those periods of development when R' is most sensitive to genetic "instruction" as well as finding more effective treatments for making heritable changes in R' expression.