2. Cytoplasmic male sterility research.

Previous research using EMS to induce cytoplasmic male sterility in corn has been reported (1). More recently Petrov and Zheleznova reported that streptomycin produces cytoplasmic male sterility (U.S. Pat. Applic. #3,594,152).

Petrov and Zheleznova used doses of 0.0005 - 100 micrograms/ml or 0.00000005 - 0.01% on germinated seed for 24 hours. In our research using streptomycin, doses of .001, .005, .01, .05, .10, .150%, and control were used. Seeds of an inbred line of corn were germinated for 30 hours at 27°C; at the end of this time some radicles had emerged. Subsets of experiments were performed; in one set the germinated seeds were placed embryo down in petri dishes on Kimpak that was saturated with the streptomycin solution. In the second set germinated seeds were completely submerged in flasks of the streptomycin solution. In another experiment dry seeds (ungerminated) were placed embryo down in Petri dishes on Kimpak that was saturated with the streptomycin solution. All these experiments were conducted for 24 hours at 25°C.

The treated material was planted by digging trenches with a hoe and placing the sprouted seeds in them, after which they were covered with soil. The procedure of putting treated seed in trenches worked quite well; one contributing factor to this success was good soil moisture.

Shortly after emergence it was noted that some of the seedlings were albino. In fact, all seedlings were albino in material that had been germinated and then completely submerged in the 0.10 and 0.150% streptomycin solution (Table 1). The albino plants did not turn green and subsequently died.

Affecting the chlorophyll was encouraging, since we are undoubtedly doing something in the cytoplasm. If we are doing something genetic remains to be determined. Apparently there have been a few genetic studies to induce cytoplasmic mutants in higher plants (1). However, the effect of streptomycin on chloroplast development was discovered by vonEuler, who found that seedlings watered with a streptomycin solution developed colorless leaves. Studies with Euglena established that growth on streptomycin led to irreversible loss of chloroplast-forming ability. It has also been reported that in algae, streptomycin is a specific mutagen for chloroplast

DNA's and that streptomycin is an effective mutagen for cytoplasmic genes (see 2).

A rather good dose response, recorded as "% of planted stand" was obtained with the various treatment procedures (Table 1). However, no sterile tassels were noted in the M_1 generation in any treatment, nor could any sterile sectors be found in the tassels. Also no observable differences were noted in the mature plants among the treatments; in fact, the surviving plants appeared quite normal. The material was self-pollinated and will be planted ear-to-row in 1973.

Table 1

Total plants, number and percent of albino plants from three streptomycin experiments (germinated seeds planted directly in field).

	Germinated seeds on Kimpak (100 planted)				Germinated seeds submerged (42 planted)				Dry seeds on Kimpak (30 planted)			
	1*	2	3	4	1*	2	3	4	1*	2	3	4
Control	82	--	--	82	38	--	--	90.5	26	--	--	86.7
.001%	78	--	--	78	35	--	--	83.3	24	--	--	80.0
.005%	90	--	--	90	27	--	--	64.3	24	--	--	80.0
.01%	87	--	--	87	30	2	6.7	71.4	23	--	--	76.7
.05%	34	--	--	34	8	4	50.0	19.0	23	--	--	76.7
.10%	29	3	10.3	29	10	10	100.0	23.8	14	3	21.4	46.7
.150%	19	9	47.4	19	16	16	100.0	38.1	13	3	23.1	43.3

*1. No. surviving plants
2. No. chlorotic plants
3. % chlorotic plants of survivors
4. % of planted stand

Research to induce cytoplasmic male sterility with EMS is continuing. As previously reported (1), male sterile plants were detected in progeny of inbred lines that had been treated with EMS. However, after crossing these sterile plants with the untreated controls the plants became fertile in the subsequent generation. This is indicative that a recessive gene for male

sterility was causing the sterility. Also a "state" of the cytoplasm (dauermodification) may have been induced by the mutagen treatment. Another possibility under investigation is that cytoplasmic mutations for sterility were induced but when they were crossed to the untreated controls restorer genes were brought in leading to fertility. This assumes that the inbred line is segregating for restorer genes. To examine this possibility, remnant seed from each treatment that showed male sterility was planted and outcrossed with one of two unrelated inbred lines. These F_1's have been self-pollinated and will be planted in order to examine this theory. This approach may be feasible since Edwardson (3) reported that genes which restore fertility to cytoplasmic male sterile corn occurred in 59.6% of Latin American varieties and that the frequency of such genes in U.S. inbreds is 10.5% and that 2.81% were segregating for restorer genes. Also the variety Golden June, the source of Texas male sterile cytoplasm, was segregating for restorer genes (4).

References

1. Briggs, Robert W. (1971) Maize Genetics Newsletter 45:13-16.
2. Sager, Ruth (1971) Cytoplasmic Genes and Organelles. Academic Press, New York.
3. Edwardson, John R. (1955) Agronomy Jour. 47:457-461.
4. Brooks, James S. (1961) Crop Sci. 1:224-226.

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1. Unusual reaction of N and T cytoplasms to H. maydis, Race T.

One maize hybrid with N cytoplasm in a 1971 experiment segregated into a 3 resistant:1 susceptible phenotypic ratio. The same hybrid with T cms had one resistant plant. Paired entries were planted with hand planters. It is possible, but not likely, that this plant resulted from a kernel intended for the adjacent plot.

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