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1. "Curing" maize of its plasmids.

Plants infected with about 30 different viruses have been reported to be cured by exposure to high temperature. Various chemicals also have been reported to differentially inactivate viruses. It has been repeatedly suggested that cytoplasmic male sterility in maize may be due to the presence of an alien virus. A very crudely designed experiment in 1959 gave evidence that maize has been "cured" of its cytoplasmic male sterility by heat shock (MNL 35:83, 1961). In 1961 and 1962 this experiment was repeated using refined seed stocks and equipment and the heat shock treatment was extended to include maize carrying a variety of plasmids and/or episomes in an attempt to "cure" them of their "infection". In addition, lines of the same material were treated with streptomycin, dl-parafluorophenylalanine, and acriflavine which are known to kill, inactivate or induce mutations in viruses and other plasmids. Both mutant forms of the plasmids and the elimination of the plasmids from the cell line (cure) were sought in the treated material. No effect was detected as a result of any treatment. These experiments contribute no evidence in support of the idea that controlling elements are due to the presence in the cytoplasm of virus-like entities in so far as their reproduction may be inhibited by external agents without killing the host cell. A brief account of these experiments is presented here for the record.

Technique: The heat treatment procedures were previously described (MNL 35:83). In 1961 and 1962 a constant temperature water bath replaced the oven., and aluminum foil pans 6 3/4 x 4 1/4 x 1 3/4 inches replaced the cardboard dishes used in 1959-60. The seeds when planted were routinely maintained for 48 hours at 30°C and then treated for 12 hours at 46°C. Following treatment they were transferred to the greenhouse and subsequently the surviving young plants were transplanted into the field.

The chemical treatment consisted of soaking dry seed for 24 hours at 30°C in the dark. The chemicals used and the concentrations used were:

streptomycin - 0.025% and 0.1% in water
dl para fluorophenylalanine - 0.0125%, 0.025% and 0.1% in water
acriflavine - 0.025% and 0.1% in water.

The soaked seeds were planted directly in the field. No phytotoxicity was noted and in most cases nearly 100% survival was obtained.

Cytoplasmic male sterility

A. Heat treatment: During 1960 an inbred line with the rare recessive leaf stripe gene - j_2 - and carrying either "T" or "S" cytoplasm following 8 backcrosses was multiplied for use in a large scale experiment. In 1961, 15,600 seeds were heat treated and 2,750 seeds served as controls. Only "T" cytoplasm was used. At flowering time, in August, only 1,988 remained from the treated group and only 728 from the control group. No effect of treatment was noted.

The 1962 experiments were conducted on fewer seeds but a much higher survival rate following transplanting was achieved. Both "T" and "S" cytoplasm were used. 2,700 "T" cytoplasm treated seeds were represented by 1,263 field survivors in August and 450 untreated seeds following transplanting gave 441 plants in the field. "S" cytoplasm stocks consisted of 900 treated and 150 untreated with 273 plants flowering from the heat treated and 148 from the non treated. No plants shed pollen in 1962 in either of these experiments.

B. Chemical treatment: 134 seeds of the same inbred stock with "T" cytoplasm used in the heat treatment were treated with each concentration of each chemical while 268 seeds were soaked in water. None of the treated or water soaked seeds produced pollen shedding plants.

Variegated pericarp

A. Heat treatment: One experiment was conducted in 1961 using seed from the mating of homozygous variegated (inbred W9 background) x $\underline{P}^{ww}$(inbred A171). The results obtained are tabulated in the table under "Homozygous Variegated".

In another experiment in 1961, 980 seeds from the mating medium variegated ($\underline{P}^{vv}/\underline{P}^{wr}$) x inbred W9 ($\underline{P}^{wr}$) were treated in the water bath, while 150 seeds were germinated and transplanted as controls. The results obtained are shown in the table under "Heterozygous Variegated".

Pericarp phenotype	Homozygous Variegated				Heterozygous Variegated			
	Control		Heat treated		Control		Heat treated	
	Total number	%	Total number	%	Total number	%	Total number	%
medium variegated	61	83.6	108	87.8	45	36.3	245	36.3
light variegated	8	11.0	4	3.3	7	5.6	43	6.4
very light variegated	0	0	1	0.8	1	0.9	1	0.1
colorless pericarp	0	0	1	0.8	66	53.2	332	49.2
red	4	5.4	9	7.3	5	4.0	54	7.9
	73		123		124		675	

There does not seem to be any consistent effect of the treatment in the case of "Homozygous Variegated" although the numbers of survivors were too small to have much significance. A slight increase in the frequency of movement of Mp away from $\overline{prr}$ is noted in the heat treated "Heterozygous Variegated" (as indicated by the increased frequency of light variegated and reds). A larger control would have been necessary to establish the difference as real.

The seed used in the heat treatment experiments in 1962 all came from the pollination of many ears of inbred W9 with the pollen from one homozygous variegated plant (in W9 background). 3,600 seeds were treated in the water bath and 600 were transplanted without treatment. The results of the analysis of the ears are shown below:

	Controls		Heat treated	
	Number of ears	%	Number of ears	%
1. Pericarp class				
medium variegated	506	94.2	1,361	93.8
light variegated	23	4.3	40	2.8
very light variegated	0	0	2	0.1
orange variegated	0	0	3	0.2
red	9	1.7	42	2.9
p^{wr}	2	0.4	3	0.2
Total	537		1,451	
2. Number kernels per red spot (one kernel and larger)	3441/1526 = 2.26		8108/3871 = 2.09	
3. Number red spots (one kernel and larger) per med. var. ear	1526/506 = 3.02		3871/1361 = 2.85	
4. Estimated freq. per 1,000 kernels of red spots of kernel size:				
1 kernel	6.1986		5.4604	
2 kernels	0.3892		0.3743	
4 kernels	0.0795		0.0789	
8 kernels	0.0670		0.0418	
16 kernels	0.0167		0.0170	
32 kernels	0.0126		0.0139	
64 kernels	0.0084		0.0062	
128+ kernels	0.0126		0.0108	

Whereas there was a slight increase in the frequency of light variegated in 1961 following heat treatment, a decrease was observed in the 1962 group. And since very light variegated and orange variegated are rare phenotypes, their appearance in only the heat treated could be ascribed to chance. The number and size of red pericarp spots on the medium variegated ears do not differ enough to warrant drawing conclusions.

B. Chemical treatments: Variegated pericarp stocks in W9 background were treated with the same chemicals, concentrations and times as the cytoplasmic male sterile stocks. A total of 1,584 ears from these treatments were analysed in the same way as the heat treated variegated stocks. These extensive data show no striking departure from that in the heat treated material and so they will not be included here.

Bronze mutable: A small progeny from a homozygous bz_1^m sh ear which had been heat treated showed no striking difference from the untreated. Both heavily and lightly mottled and stable bronze ears were present in both groups in about equal numbers. No detailed analysis of t he spotting frequency or distribution was undertaken.

Dotted: The only provocative observations involve the acriflavine treated progeny of a homozygous a_1 Dt ear in inbred W9 background. Among 230 selfed ears coming from one selfed ear, 209 were normally dotted, 6 were segregating 3 dotted: 1 non-dotted and 15 showed a few kernels without dots while other kernels on the same ear showed all gradations of dots up to the usual level in this line. Through an oversight in the field, the untreated control material was not hand pollinated so no conclusions can be drawn.

Robert I. Brawn

2. Dark variegated pericarp.

Last year it was reported that dark variegated pericarp occasioned a coarse (earlier) pattern of Ds breaks than the standard medium variegated when both were used as males on Ds testers. In 1962 similar crosses were made onto the progeny of one selfed ear of homozygous Ds. Again the visible pattern of Ds breakage was coarser when the male was dark variegated than when the male was medium variegated. The difference has not yet been scored qualitatively. These further observations support the hypothesis that the dark variegated phenotype results from a change of state of Mp in the direction of a lower dosage than that of the standard Mp of medium variegated.

Medium variegated (P^{vv}/P^{wr}) when tested showed the expected 1/2 kernels with Ds breaks and 1/2 without breaks while homozygous medium variegated produced nearly all kernels with Ds breaks. However, homozygous dark variegated gave 20 to 30% kernels without Ds breaks. Likely the kernels on the Ds tester without breaks are the result of the loss of Mp from P^{rr}. This is consistent with the high frequency of red ears in the progeny of dark variegated reported last year. In