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Ram S. Verma

2. Induction of mutations in maize with mitomycin C.

Mitomycin C (MC) is a mutagen in some strains of E. coli in which it also increases the frequency of recombination (Iijima and Hagiwara, 1960). In Ustilago and Saccharomyces, Holliday (1964) indicated that MC is nonmutagenic in these organisms although it does increase the frequency of mitotic crossing over. The purpose of the present study was to examine the effect of MC on mutations in maize.

In the first experiment, homozygous triple dominant stock of "Seneca - 60" (S-60), Yg_2, Sh_1, and Wx, and homozygous recessive No. 9 tester were used. The experiment was carried out in the field. Before crossing, tassels of the S-60 male parent were treated with 0.0005% MC for 6 hrs. The time for treatment was 4-6 days before pollen shedding. The leaves surrounding the shoot were slit open with a razor blade. The tassel branches individually were imbedded tightly in cotton. Leaves were closed and wrapped with masking tape. A 0.0005% solution of MC was injected into the cotton with a hypodermic syringe until saturation was reached (about 20-25 ml per tassel). The cotton and the masking tape were removed after 6 hrs. of treatment. During flowering, the pollen from treated plants was collected and used to cross on to the No. 9 tester plants. Mutation frequencies of the sh_1 wx loci were determined from the F_1 seeds.

The total mutation frequency (Table 1) induced by MC was 0.123% for the sh_1 locus and 0.090% for the wx locus, as compared to 0.012% for shrunken and for waxy in the control, an increase of almost X10.

Table 1. Frequency of mutants obtained in a cross of $\underline{yg_2}\ \underline{sh_1}\ \underline{wx}$ X $\underline{Yg_2}\ \underline{Sh_1}\ \underline{Wx}$ following MC treatment.

Treatment	Population (no. of seeds)	No. of mutants			Total mutants			
		Single $\underline{sh_1}$	Single $\underline{wx}$	Double $\underline{sh_1}\ \underline{wx}$	sh_1	sh_1 frequency %	wx	wx frequency %
Control	7877	1	1	0	1	0.0126	1	0.0126
0.0005% MC	8915	4	1	7	11	0.123	8	0.0897

In the second experiment, F_1 seeds (No. 9 tester X S-60) were soaked in one of three concentrations of MC (0.0005, 0.001 or 0.004%) at 22 $\pm$ 2°C (room temp.) for 12 hrs. The numbers of seeds used for each treatment are shown in Table 2. In order to facilitate uniform absorption during the treatment, the flasks containing the seeds were shaken every 30 minutes.

The seeds were germinated on moist filter paper in Petri dishes at room temperature. Three days after treatment, six root tips were selected randomly from each treatment and fixed for cytological studies. The percentage of germination and the root length were also scored at that time. The germinated seeds were sown in the greenhouse. Seedling height was determined from 14 day old seedlings grown in the greenhouse; then they were transplanted to the field. Viable chlorophyll mutations of M_1 plants were scored in the field during the entire life cycle of plants.

The effects of MC were studied on seed germination, seedling growth, root growth, mutation rate and chromosomal breakages. In Table 2, seeds treated with one of these three concentrations showed that there was no effect on germination as compared to the control, but survival decreased as the concentration was increased. There was no significant difference in the mean height of seedlings in solutions of 0.0005% and 0.001%, but in the 0.004% solution, the mean seedling height was significantly lower. Seedling height and root length were drastically

Table 2. Effect of the treatment of F_1 maize seeds (No. 9 x S-60) with MC for 12 hrs. at room temperature on germination, seedling growth, and viable chlorophyll mutation rate.

Treatment	Condition	No. of seeds	% of germination	Root length (3 days after treatment)		Plant height (14 days)		Survival after 30 days (%)	Viable chlorophyll mutation (M_1 plant basis)	
				Mean length (cm)	% reduction of control	Mean height (cm)	% reduction of control			
Control	H_2O	100	94	6.6	-	24.7	-	100	0	-
MC	0.0005%	150	88.7	4.6	30.3	20.7	16.2	90.2	14	10.5%
MC	0.001%	150	92	4.2	36.4	21.2	14.2	87.7	19	13.7%
MC	0.004%	200	91	2.7	59.1	17.9	27.5	85.7	23	12.6%

Table 3. Effect of the treatment of F_1 maize seeds (No. 9 x S-60) with MC for 12 hrs. at room temperature on chromosome breakage, as studied in first root tip mitosis 3 days after treatment.

Treatment	Concentration	No. of anaphase cells scored	No. of abnormal anaphases	% of abnormal anaphases	No. of bridges and pseudo-chiasmata	No. of fragments	No. of lagging chromosomes
Control	H_2O	413	3	0.7	2	1	2
MC	0.0005%	233	20	8.58	15	14	1
MC	0.001%	242	31	12.81	14	26	5
MC	0.004%	195	49	25.13	20	43	15

reduced at the high concentrations. The root length in the highest concentration (0.004%) produced at 59.1% reduction over the control value.

The various types of cytological abnormalities of root tip mitoses and their frequencies are shown in Table 3. Anaphase bridges and fragments occurred frequently in the treated materials, although very few aberrations were found in the control material. The frequency of chromosomal aberration was proportional to the concentration. The reduction of root length as well as the percentage of abnormal cells showed a correlation with the concentration.

The most striking phenotypic changes in the MC-treated M_1 plants were yellow, albino, and pale-green longitudinal stripes of various sizes in the leaves. The frequency of viable chlorophyll mutations is shown in Table 2. One albino plant was observed in this experiment. No chlorophyll mutants were found in the control. These experiments suggest that MC is also a mutagen in maize.

References:

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M. S. Lin

3. The effects of hydroxylamine sulfate on the nuclear cycle and endoreduplication in maize root tips.

Two experiments have been conducted to study the effects of hydroxylamine sulfate (HAS) on the nuclear cycle and endoreduplication. Intact roots of "Seneca - 60" were exposed to ^{3}H-thymidine (1 uc/ml final concentration) for 30 min., followed by a 0.05% HAS treatment for 2 hrs. After washing, the intact roots were returned to the germination chamber (24±1°C) for further growth and fixed at 2-hour intervals up to 32 hrs. post-treatment. Autoradiographs were prepared according to the schedule of Douglas (MGCNL 42: 175-178, 1969) and from Feulgen squashes of this material. For each treatment and control, the mitotic index and prophase labelling index were determined.