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INTERACTION OF FORMALDEHYDE AND X-RAYS ON MUTATION PRODUCTION IN DROSOPHILA MELANOGASTER

FORMALDEHYDE, when mixed into the food, is known to act as a strong mutagen for *Drosophila melanogaster*. However, the conditions for its action are more stringent than for most other mutagens. For, although formaldehyde has been shown to penetrate all germ cell stages of larvae and adults of both sexes¹, only the auxocyte cells, *i.e.*, early spermatocytes, of male larvae yield to its mutagenic action. Germ cells of female larvae and adults do not respond to the treatment².

During the course of our investigations on formaldehyde mutagenesis, yellow flies were repeatedly recovered among those emerging from treated larvae. This led us to test the possibility that development on formaldehyde might result in selection of yellow mutations. Accordingly, we compared the frequency of lethals and mutations at the yellow locus induced by X-rays in treated larvae which completed their post-irradiation development either on basal medium or formaldehyde containing basal medium. The results of this investigation revealed an interesting deviation from the sexspecificity of formaldehyde mutagenesis and an interaction of X-ray produced pre-mutational lesion

with formaldehyde in the food. We briefly report the results in this communication.

For tests on the induction of sex-linked recessive lethals by combination of X-ray and formaldehyde treatments, $24 \pm 1.5 \,\text{hr}$ old Oregon-K larvae were irradiated with 5 kR of 50 KV unfiltered X-rays from a Philips therapeutic machine (exposure 2 minutes at 15 cm from source). The irradiated larvae were allowed to complete their development either on basal medium (X) or on basal medium containing 0.18% formaldehyde (XFH). A third set of larvae were raised on formaldehyde food without irradiation (FH). The frequency of lethals was determined in both males and females by the Muller-5 method. The lethal frequency in male larvae receiving combined treatment was marginally more than the additive value of the two separate treatments (X 0.90%; FH 8.08%; XFH 9.73%). The difference, however, was not significant. In females, where formaldehyde alone is known not to induce mutations, the combination treatment did not show any increase over X-ray treatment (X 0.86%; FH 0.18%; XFH 0.70%). The 0.70% lethals of combined treatment originate therefore from X-ray treatment alone.

A completely different picture emerged when mutations at the yellow locus were looked for. In this experiment, the X-ray and formaldehyde treatments were given exactly as in the previous experiment. Following treatment, emerging males were mated to attached-X virgin females of the constitution y v f (yellow, vermilion, forked) and treated virgin females were mated to yellow males. The progeny obtained from these matings was examined for yellow flies. There was an over ten fold increase of yellow mutations in irradiated flies which had completed subsequent development on formaldehyde food, over those which had been reared on basal medium. An increase of a similar magnitude was also obtained in treated females, where formaldehyde treatment itself, in conformity with earlier observations, was shown to be ineffective. Specifically, there were 2 yellow mutations among 11336 tested chromosomes in X-ray treatment alone as compared to 19 among 9794 chromosomes in XFH treatment; the increase is statistically significant.

Since formaldehyde alone does not induce mutations in female larvae, the reason for enhanced mutation frequency will have to be looked for at the level of realization of mutants rather than at the level of initial X-ray induced lesions. One possible explanation of this increase in combined treatment could be that secondary steps which lead to restoration of the X-ray induced pre-mutational

lesion back to normalcy on basal medium are blocked by formaldehyde in the food. When confirmed, it will endow formaldehyde with an unusual kind of specificity in blocking repair/recovery of alterations at the yellow locus but not affecting lethal mutations on the same chromosome. It will also be interesting to see whether the increase observed for yellow mutations extends to other visible markers on the X-chromosome. This is now under investigation.

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MUTAGENIC ACTION OF NITROSO METHYL UREA IN PEARL MILLET

Hybrid Bajra (Pennisetum typhoides), a widely cultivated millet, is losing popularity in recent years, because of its susceptibility to several diseases. Seeds of Tifton 23 A and BIL-3B, the female and male lines, respectively, of Hybrid Bajra-1 were treated with N-nitroso-N-methyl urea (NMU) to understand the mutagenic action and also to isolate disease resistant types, if any, in later generations. The present note describes the preliminary cytogenetical effects.

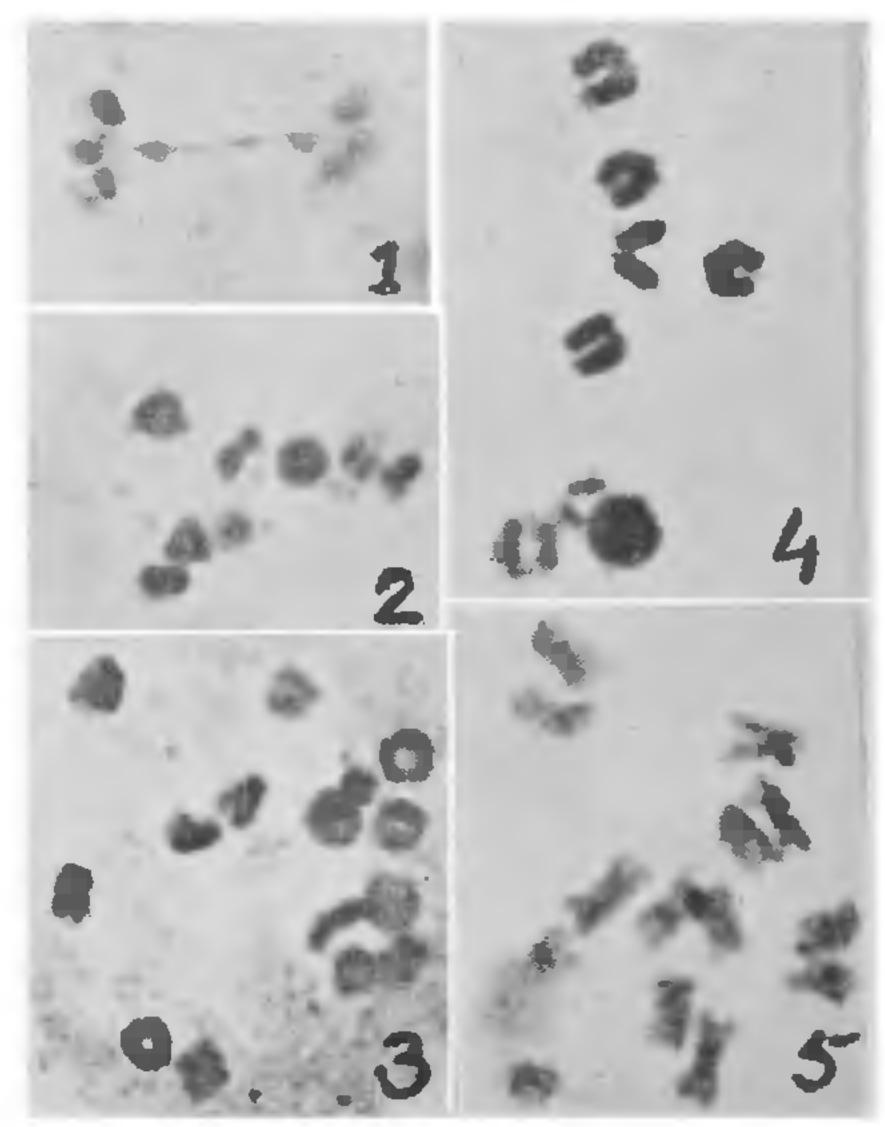
After pre-soaking for 9 hr in water, lots of 300 seeds each were treated with 0.005, 0.010 and 0.020% aqueous solution of NMU for 4 hr. Observations were recorded on germination, seedling height, mitosis, meiosis and M_2 chlorophyll mutation frequency and spectrum. The results are presented in Table I.

The data indicate that there is a progressive reduction in germination and seedling height with the increase in mutagen concentration and 0.02% NMU treatment proved to be almost lethal. The

LD 50 for both the strains was found to be around 0.015%, while the reduction in seedling height at a comparable dose was more in the case of BIL-3B than in 23-A.

Observations on mitosis recorded after fixing the roots at different intervals showed that the frequency of dividing cells dropped soon after the treatment. However, the root growth was restored and a high mitotic index was obtained with increase in post-treatment period. Besides inhibition of mitosis, stickiness, clumping and fragmentation of chromosomes was observed.

Meiotic preparations of several M_1 plants indicated abnormalities like lagging chromosomes, anaphase bridges (Fig. 1), unequal anaphase



Figs. 1-5. Meiotic stages. Fig. 1. Anaphase. bridge. Fig. 2. Chromosome fragmentation. Fig. 3. Cell with fourteen bivalents. Fig. 4. Normal cell with seven bivalents. Fig. 5. PMC with 14 mitotic chromosomes.

TABLE I

Treatment	Germination			Seedling height (cm)		Frequency of chlorophyll mutations in BIL-3B			
						No. of M ₂ families No. of M ₂ plants			
		23-A	BIL-3B	23-A	BIL-3B	scored	segregating	scored	Mutants %
Control		65	65	17-4	23 ·6	30	• •	3020	-
0.005%		5 6	61	17.4	15-5	31	21	3271	2-48
0.010%		49	54	13-1	14 - 5	30	11	3625	1 · 17
0.020%	••	2	1	0.7	0.5	4	4	303	2.95