

Differential Response of Some Tomato Genes to Base-specific Mutagens

A NUMBER of chemicals are now known which affect specific bases of the DNA molecule¹. These include the base analogues (such as fluorodeoxyuridine) and others like hydroxylamine and hydrazine which react mainly with cytosine and thymine respectively under particular conditions of treatment. The action of many of these chemicals on chromosomes and genes in higher organisms has not been tested extensively, but some reports are available which show that they induce localized damage in the nucleus²⁻⁴. We have observed a differential reaction of tomato chromosomes when pollen mother cells are treated with hydroxylamine⁵. The main finding was that a few of the chromosomes, particularly chromosome 2, were much less damaged than other members of the complement. These experiments with hydroxylamine and a number of other chemicals and X-rays have now been extended to compare the spectrum of mutations induced by them; the present communication relates to some of the results obtained.

The different treatments, details of which are presented in Table 1, were given to seeds (500 in each case) pre-soaked in water for various durations. The pH's of the freshly-prepared solutions were adjusted in a manner described elsewhere⁶. The observations on induced mutations were recorded mainly on seedlings which are easier to handle in large numbers. Also, a number of gene mutations affecting seedling characters are known to give such characteristic phenotypes that the induced variation can easily be scored for them⁶.

These observations make it clear that hydrazine and hydroxylamine induce mutations at a number of loci with very different frequencies. These loci, moreover, are not randomly distributed in the genome. A number of them (*d*, *aw*, *wo*) are known to be located on one particular chromosome (chromosome 2). It should be emphasized that although hydroxylamine failed to induce mutations at these loci, the overall mutation rate with this chemical was higher than that with hydrazine, as shown by the frequency of lethal seedlings and other variation not included here because it could not easily be classified in relation to any of the known genes. Also, for comparable concentrations, hydroxylamine is found to induce significantly greater chromosomal damage and reduce germination percentage to a much greater extent (see Table 1). Ethylmethanesulphonate, which is known to react with several bases⁷, differs from both in inducing chlorophyll deficiencies of various kinds, but apart from this, it appears to correspond in its action more to hydroxylamine than to hydrazine.

The observations, and the cytological analysis reported earlier, suggest that a number of loci, particularly on chromosome 2, may be characterized by an inhomogeneity in the distribution of adenine-thymine and guanine-cytosine base pairs in their DNA. Differences in base composition of this nature, if confirmed, may prove to be of considerable interest, for they may be of significance in relation to what Rick⁸ has described as the asymmetrical distribution of genes on tomato chromosomes, with chromosome 2 having nearly 25 per cent of all the mapped genes located on it. No satisfactory explanation for this asymmetry has so far been available. Further analysis using techniques based on autoradiography and ultracentrifugation is in progress.

We thank Dr. M. S. Swaminathan for his kind interest in this work.

H. K. JAIN
R. N. RAUT

Botany Division,
Indian Agricultural Research Institute,
New Delhi.

¹ Freese, F., *Molecular Genetics*, Part I, 207, edit. by Taylor (Academic Press, New York, 1963).

² Somers, E. C., and Hsu, T. C., *Proc. U.S. Nat. Acad. Sci.*, **48**, 937 (1962).

³ Natarajan, A. T., and Upadhyay, M. D., *Chromosoma*, **15**, 156 (1964).

⁴ McLeish, J., *Heredity*, **8**, 385 (1954).

⁵ Jain, H. K., and Raut, R. N., *Experientia*, **21**, 322 (1965).

⁶ Rick, C. M., and Butler, L., *Adv. Genet.*, **8**, 267 (1956).

⁷ Kreig, D. R., *Genetics*, **48**, 561 (1963).

⁸ Rick, C. M., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1515 (1959).

CYTOLOGY

Cytoplasmic Deoxyribonucleic Acid-containing Bodies in Amoebae

EVIDENCE has been presented, from investigations involving techniques of nuclear transfer and injections of cytoplasm, that certain cellular properties of *Amoeba proteus* and *Amoeba discoides*, such as nuclear diameter and sensitivity to streptomycin, are influenced by self-replicating cytoplasmic hereditary determinants¹⁻⁶. The finding of DNA- and RNA-containing bodies in the cytoplasm of *Amoeba proteus*⁷⁻⁹, followed by the demonstration that these bodies are self-replicating within the cell^{8,9} and that they show a certain degree of interaction with the nucleus of amoeba⁸, has stimulated interest in the possibility that they contain the physical basis of this cytoplasmic inheritance⁶.

Cells of the strain of *Amoeba proteus*, τ_1P , and the strain of *Amoeba discoides*, τ_1D , used in the nuclear transfer

Table 1

Treatment	M ₁ seed germination (per cent)	Dwarfs (<i>d</i>)	Frequency of different mutant seedlings (Percentage of M ₁ seedlings)			Chlorophyll deficient	Lethals †
			Without anthocyanin (<i>aw</i>)	<i>d</i> + <i>aw</i> *	Woolly (<i>wo</i>)		
Hydrazine (pH 8.5)							
(i) 0.1 M, presoaked for 16 h, treated for 20 h	40.8	1.96	0.98	0.49	0.98	—	0.98
(ii) 0.1 M, presoaked for 16 h, treated for 30 h	40.0	0.50	—	1.00	0.50	—	1.50
(iii) 0.05 M, presoaked for 20 h, treated for 12 h	63.0	—	0.95	0.63	0.63	—	—
(iv) 0.00 M, presoaked for 16 h, treated for 30 h (Control)	64.8	—	—	—	—	—	—
Hydroxylamine (pH 7.0)							
(i) 0.05 M, presoaked for 16 h, treated for 20 h	17.4	—	—	—	—	—	5.74
(ii) 0.025 M, presoaked for 20 h, treated for 12 h	46.4	—	—	—	—	—	3.87
(iii) 0.012 M, presoaked for 20 h, treated for 30 h	45.9	—	—	—	—	—	3.26
(iv) 0.00 M, presoaked for 16 h, treated for 20 h (Control)	76.6	—	—	—	—	—	—
EMS (pH 7.0)							
(i) 0.03 M, presoaked for 16 h, treated for 20 h	47.6	—	—	—	0.42	0.84	5.45
(ii) 0.03 M, presoaked for 16 h, treated for 30 h	45.4	0.44	—	—	—	1.32	7.92
(iii) 0.00 M, presoaked for 16 h, treated for 30 h (Control)	80.8	—	—	—	—	—	—
X-ray (dry seeds)							
(i) 20,000 r.	64.2	—	—	—	—	—	0.21
(ii) 30,000 r.	53.8	—	—	—	0.28	—	0.82
Control	86.2	—	—	—	—	—	—

See Rick and Butler (ref. 6) for a description of the mutant phenotypes. Control mutant stocks for comparison were made available by Dr. L. Butler, whose generous gifts of seeds are gratefully acknowledged.

* The simultaneous induction of these double mutants of linked genes is obviously of considerable significance.

† These variously deformed seedlings do not survive beyond the cotyledonary or 1-2 true leaf stage. A statistical analysis shows that hydroxylamine and hydrazine induce them with significantly different (1 per cent level) frequencies.