Editorials

with that of individuals with "Aso-Volcano Disease". They carried out a systemic survey on disorders in children with mottled teeth, particularly with reference to electrocardiographic changes. Takamori established a classification of mottled enamel which permits an exact description of every tooth of a denture affected by dental fluorosis.

A future issue of "Fluoride" will present details of Takamori's important work.

THE THIRD CONFERENCE OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

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1969

CYTOGENETIC EFFECTS OF HYDROGEN FLUORIDE ON PLANTS

by

Aly H. Mohamed
Kansas City, Missouri

Fluorocompounds, either organic or inorganic, are considered to be potential pollutants. These compounds are by-products of various industrial processes, such as the production of phosphate-fertilizers, ceramics and certain metals such as aluminum.

Gaseous hydrogen fluoride is one of the most phytotoxic of the halogen compounds occurring as air pollutants (1). According to Hill et al. (2) fluoride gas in the atmosphere may cause severe damage to plants even when the concentration is below the level required to produce visible morphological symptoms.

The objective of this report is to correlate the findings of Mohamed et al. (3) and of Mohamed (4) in tomato plants with those in maize.

Materials and Methods

In both experiments, on tomatoes and on maize, the plants were fumigated with fluoride gas with a concentration of about 3 $\mu g/m^3$. Temperature, humidity, and light quantity and quality were closely controlled with a photoperiod of 13 hours for the tomato and 14 hours for the maize plants. Both experimental treatments were run for 12 days with some plants removed from the fumigation chambers after an initial period of four days, and equal numbers of plants removed each two days following, until the final ones were taken out at the end of the 12 day period. Thus, there was a total of five different periods of treatments for the tomatoes and the maize. Control runs were always made simultaneously with treatment runs. A detailed method was given for the tomato plants in the two previous papers (3 and 4). However, with respect to the maize experiment, microsporocyte samples collected from treated and control plants were killed and fixed separately in 2:1 ethyl alcohol-propionic acid and stored in a freezer until examined in acetocarmine smears.

Results

It was demonstrated in tomatoes by Mohamed et al. (3) that a low concentration of HF gas in the atmosphere, which did not induce any

From the Department of Biology, University of Missouri, Kansas City, Missouri 64110.

visible injury, was capable of inducing chromosomal aberrations in the somatic and meiotic chromosomes of the tomato plants. Table 1 shows a positive correlation between the frequency of chromosomal aberrations and duration of treatment in the smears of young leaf. These aberrations were mainly anaphase bridges with or without fragments or fragments by themselves. The formation of bridges was attributed to the primary effect of HF, as well as to chromosomal rearrangements. The primary effect of HF, which is stickiness of the chromosomes, disappeared completely when the "recovered" tomato plants were studied for the presence of observable cytological abnormalities in leaf smears. These recovered plants did not show any noticeable chromosomal aberrations.

TABLE 1

Percentages of Cells with Chromosomal Aberrations in Anaphase of Leaf Smears

Treatment in days	No. cells	Bridges	Fragments	Bridges + Fragments	Total aberrations
		Treated			
Control	300	2.0	----	----	2.0
4	366	24.0	4.4	6.8	35.2
6	400	31.0	4.0	4.5	39.5
8	189	31.7	6.3	14.7	52.7
10	232	37.5	8.6	8.6	54.7
12	152	36.4	11.7	20.1	68.2
		Recovered			
Control	122	----	----	----	----
4	233	----	----	----	----
6	438	----	----	1.4	1.4
8	234	----	----	----	----
10	223	----	----	.9	.9
12	292	----	----	.7	.7

In meiosis of the fumigated tomato plants (C_o), Mohamed et al. (3) had observed a significant trend toward a higher percentage of chromosomal aberrations with an increase in the duration of the treatment (Table 2). The formation of bridges with or without fragments and fragments alone in either the first or the second anaphase were attributed to the physiological effect of HF, as mentioned in mitosis, to the presence of inversions, or to both. The recovered plants, however, showed no bridges without fragments, indicating that the effect of HF

did not persist. Thus the conclusion was reached that HF gas was able to produce inversions and probably other structural changes such as translocations, duplications and deficiencies.

TABLE 2

Percentages of Cells with Chromosomal Aberrations in Anaphases and Telophases of the First and Second Meiotic Division

Treatment in days	Anaphase I + Telophase I: No. cells	Bridges	Fragments	Bridges + Fragments	Total aberr.	Anaphase II + Telophase II: No. cells	Bridges	Fragments	Bridges + Fragments	Total aberr.
					Treated					
Control	300	13	2.0	----	2.3	300	.3	---	---	.3
4	160	4.4	6.3	.6	11.3	329	1.5	7.3	.6	9.4
6	300	4.7	4.7	1.7	11.1	110	6.4	8.1	.9	15.4
8	500	3.2	8.9	.4	12.5	490	4.3	7.3	.8	12.4
10	216	30.6	8.8	33.3	72.7	108	13.0	7.4	8.3	28.7
					Recovered					
Control	113	----	3.5	----	3.5	215	----	.5	---	.5
4	145	----	5.5	4.8	10.3	134	----	14.9	3.0	17.9
6	237	----	23.6	4.6	28.2	255	----	23.1	3.9	27.0
8	471	----	20.6	8.1	28.7	195	----	24.1	10.7	34.8
10	212	----	25.5	11.3	36.8	62	----	25.8	3.2	29.0
12	219	----	19.18	4.57	23.75	155	----	15.44	2.20	17.64

When seeds from the C_o tomato plants were planted, they produced a number of abnormal phenotypes, the same as, or similar to, known mutants (Table 3). Also meiotic studies on the C_1 generation (progeny of C_o) showed a positive correlation between the treatment and the frequency of chromosomal aberrations (Table 4).

Since the maize microsporocytes were not collected immediately after fumigation but 7-10 days before the tassel tip first begins to make its appearance, the meiotic chromosomes did not show the primary effect of HF i.e., stickiness of the chromosomes. While these studies have not been completed, the available cytological data confirms those previously reported in the tomato plant experiments.

Pachytene studies indicate the presence of translocations, inversions either peri-or paracentrics, as well as deficiencies or duplications. In some cases asynaptic chromosomes were observed forming openings

TABLE 3

Percentages of Germination and Phenotypic Abnormalities Observed in the C_1 Generation

Treat-ment	No. Seeds	Germin-ation	3 cots	4 cots	Deformed cots	Fasciated petioles	Wiry	Double stalk	Plumu-less	Dwarf	Total abnormals
Control	154	77.3	.9	.9	4.7	----	---	.8	---	.8	8.1
4 days	546	80.8	1.2	--	9.0	5.4	---	.2	1.2	2.6	19.6
6 days	570	82.9	1.6	.2	6.3	10.1	---	3.8	---	2.9	24.9
8 days	329	79.7	1.2	--	10.2	8.5	.5	2.2	.5	5.0	28.1
10 days	393	84.5	3.0	.3	10.3	5.3	3.3	2.7	2.0	3.0	29.9
12 days	335	77.9	2.5	.8	8.6	7.4	---	5.3	2.9	4.5	32.0

TABLE 4

Percentages of Cells with Chromosomal Aberrations in Anaphase and Telophase of the First and Second Meiotic Division of the C_1 Generation

	Anaphase I + Telophase I						Anaphase II + Telophase II						
Treat-ment	No. cells	Fragments 1	Fragments 2	>	Bridges + Fragments	Total Aberr.	No. cells	Fragments 1	Fragments 2	>	Bridges + Fragments	Total Aberr.	Gross Total Aberr.
Control	117	---	---	---	---	0.0	320	.6	---	---	.5	1.1	1.1
4 days	393	2.4	2.4	---	2.0	6.8		6.6	4.6	---	2.6	13.8	20.6
6 days	442	1.3	.4	---	3.1	4.8	217	1.3	1.3	---	2.3	4.9	9.7
8 days	772	1.8	.6	.1	4.1	6.6	771	2.8	1.6	1.6	2.5	8.5	15.1
10 days	372	2.8	---	---	5.7	8.5	303	1.9	1.6	---	6.2	9.7	18.2
12 days	287	2.9	.9	3.8	11.6	19.2	184	---	---	.5	8.1	8.6	27.8

along the synaptic plane of the two homologues. This can be attributed to heterozygous inversions as reported by McClintock (5) and Maguire (6) in corn. Such failure of reverse synapsis at pachytene may be expressed as nonhomologous rod pairing or as complete pairing failure. In a very few cases the breakage positions were determined (Figures 1-4). In these figures the breakage for the pericentric inversion was in chromosome one at L. 18 and S. 21. In the paracentric inversion it was in chromosome 7 at L. 02 and .18. Two heterozygous reciprocal translocations were also identified. One involved chromosomes 1 and 2 with the breakage positions at 1S. 01 and 2L. 31. The second translocation involved chromosomes 5 and 10 with the breakage positions at S. 13 and L. 31 respectively. Fig. 5 shows a ring of four chromosomes in diakinesis due to a heterozygous reciprocal translocation involving two chromosomes.

Some metaphase I stages showed precocious separation of the homologous chromosomes, (Fig. 6 and 7). It can be seen from these two figures that the two homologues either pass to the same pole or to the opposite one. This precocious separation could be attributed to reduction in chiasma frequency due to heterozygous structural changes such as deficiencies or duplications (Fig. 8).

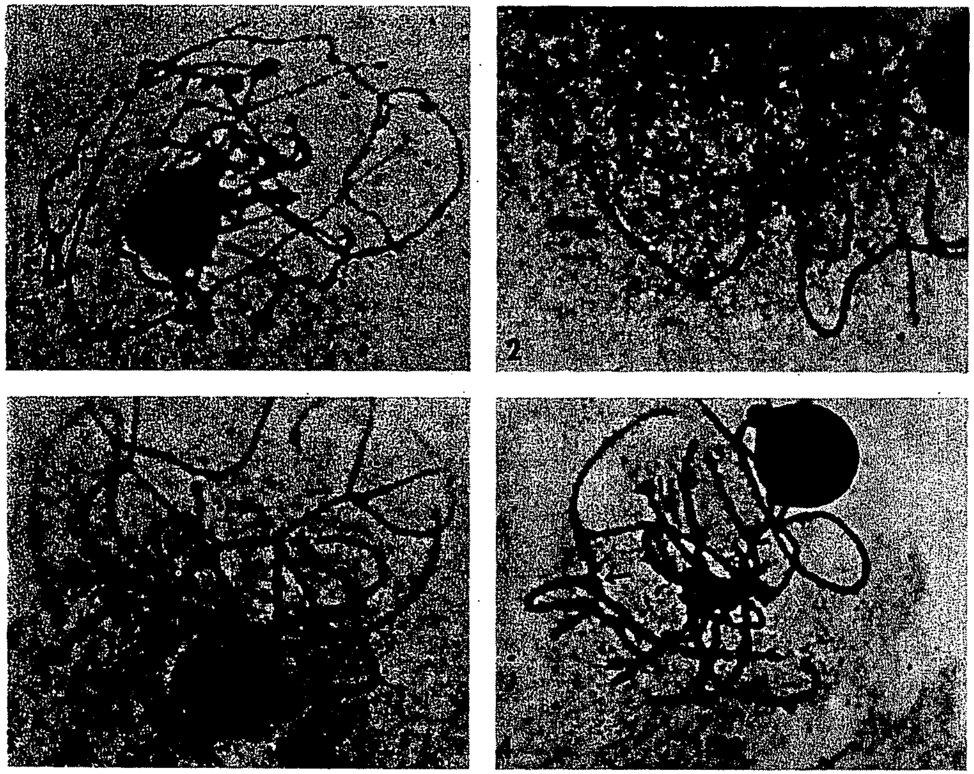
The formation of bridges plus fragments or fragments by themselves, either in the first or the second anaphase (Fig. 9-11) are an indication of the occurrence of crossing over in heterozygous paracentric inversions.

Discussion

From the data presented in this report it is apparent that hydrogen fluoride gas at a concentration below that required to induce visible injuries was able to produce permanent hereditary changes in tomato as well as in maize plants. The chromosomal aberrations induced by HF may be attributed to the action of fluoride on DNA synthesis, not through energy as in radiation, but through a chemical one.

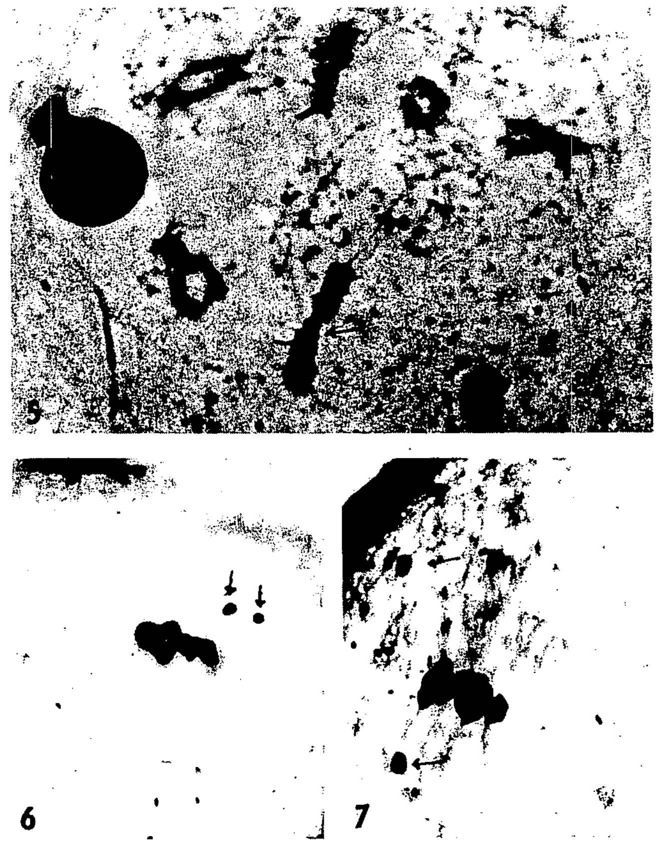
Taylor et al. (7) assumed that fluorodeoxyuridine (FUdR) was able to induce breakage in root tip chromosomes of Vicia faba through inhibiting the synthesis of thymine in DNA replication. The same results were obtained by Ahnstrom and Natarajan (8) and gave evidence for the DNA breakdown due to lowering the optimum concentration level of thymidine triphosphate through the inhibition of the activity of the enzyme thymidylate synthetase. Consequently this inhibits the biosynthesis of thymidine nucleotide and causes chromosomal breakage by means of reversing the DNA polymerization reaction. Thus, the damage to the mitotic chromosomes is caused by the enzyme DNA polymerase at the "S" phase of the caryiokinetic cycle of the cell.

The essential mechanism of chromosomal replication in mitosis is similar to that of meiosis. Therefore, the present studies suggest that the action of HF has to be in the "S" phase by blocking directly or indirectly,



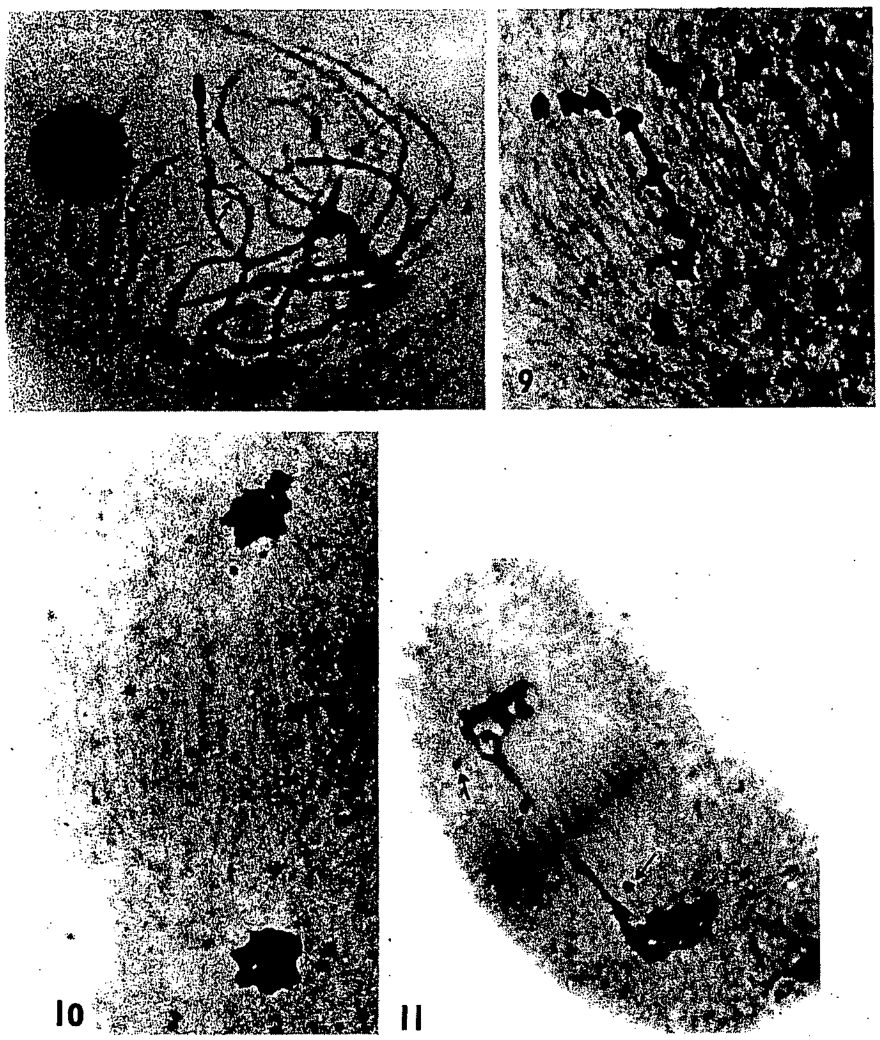
Maize Microsporocyte Smears in Pachynema from Treated Plants

Fig. 1: A heterozygous pericentric inversion (indicated by the arrow). Fig. 2: A heterozygous paracentric inversion (indicated by the arrow). Fig. 3: A heterozygous reciprocal translocation (indicated by the arrow) involving chromosomes 1 and 2. Fig. 4: A heterozygous reciprocal translocation (indicated by the arrow) involving chromosomes 5 and 10.



Maize Microsporocyte Smears in Pachynema from Treated Plants

Fig. 5: Diakinesis showing eight bivalents and a twisted ring of four chromosomes (indicated by the arrow). Fig. 6: Metaphase I showing the two homologous chromosomes passing precociously to the same pole. Fig. 7: Metaphase I showing the two homologous chromosomes passing precociously to the opposite poles.



Maize Microsporocyte Smears in Pachynema from Treated Plants

Fig. 8: Pachytene stage showing asynaptic regions (indicated by the arrows). Fig. 9: Anaphase I bridges with fragments (indicated by the arrows). Fig. 10: Telophase I showing a lagging fragment. Fig. 11: Telophase II, one cell, showing a bridge and fragments (indicated by the arrows).

the replication of the DNA molecule.

Summary

Studies on the effects of HF on meiotic chromosomes of tomatoes indicated a trend toward a higher frequency of chromosomal aberrations with an increase in the fumigation period. It was indicated that HF was capable of inducing paracentric inversions with the possibility of the induction of deficiencies, duplications or even translocations. The progeny obtained from the treated plants produced a number of abnormal phenotypes, the same as, or similar to, known mutations. Further studies in maize microsporocytes for plants treated with HF confirmed the cytological results obtained in tomatoes with clear evidence of the occurrence of inversions, translocations and deficiencies. These results suggest that HF seems to affect primarily the DNA molecule by blocking its replication, probably through its action on the enzymatic systems.

Acknowledgment

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