CYTOGENETIC EFFECTS OF GASEOUS FLUORIDES ON GRAIN CROPS

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SUMMARY: Atmospheric pollution of fluoride from a non-ferrous industrial plant has been studied for its effect on the frequency of chromosome aberrations in root tips and shoot tips of wheat and barley. The anaphase-test was used. The percentage of mutations in the meristematic cells of plants growing in polluted areas was 2-6 times higher than in the control and the spectrum of chromosome abberations showed changes. The testing of hydrogen fluoride (HF) for its mutagenic activity by fumigation of barley seedlings showed that the mutation rate was linear with dose. It was found that the cytogenic effects of gaseous fluoride on grain crops was correlated with the fluoride content in plant tissue.

Key words: Environmental pollution; Gaseous fluorides; Grain crops; Mutagenic effects; Ukraine.

Introduction

The interest in environmental mutagenesis has strengthened considerably following understanding of the broad overlap between mutagens and carcinogens. Also alterations in environmental mutagenicity lead to increases in the mutability of living organisms. Little however is known concerning mutagenic effects of gaseous fluoride, in particular fluorine containing emissions from industrial plants.

Previous studies have shown that grain crops in areas surrounding fluoride-emitting industries and in fumigation experiments have been adversely affected in growth rate, apparent photosynthesis, respiration rate, and total yield of plants. There is evidence that if enough metabolic sites are affected or the inhibition of a major pathway becomes sufficiently great, alterations in the genetic material can occur. That is why it is suggested that fluoride in its gaseous form may be a mutagen. Moreover, Mohamed observed chromosomal aberrations in tomato and corn, and in onion roots after fumigation with HF or treatment with sodium fluoride solutions (1).

Objectives of this study were to determine whether gaseous fluorides can induce chromosome aberrations in meristematic cells of plants. Thus, we have considered:

1. The mutation rates in grain crops in zones of chronic pollution from fluorine-containing industrial emissions and the spontaneous background level of the mutations.
2. Testing gaseous fluorides for their mutagenic activity by fumigation of barley seedlings in growth chambers.
3. The relationship between the chromosome aberrations and fluoride content in plant tissue.
4. The spectrum of chromosome aberrations in root tips and shoot tips of wheat and barley.
5. A comparison of the mutability in wheat and barley.

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Materials and Methods

The species of grain crops chosen for this study were winter wheat (Odesskaja semi-dwarf) and spring barley (Zernogradskii 73). The crops were sampled in the vicinity of the biggest non-ferrous metallurgy plant in Europe (Nikopo, Dniepropetrovsk region in the South-East of the Ukraine). The area used for control was situated 60 km from this plant and was free of industrial pollution of any type. The control area was agriculturally similar to the study area. It was possible at both sides to collect wheat and barley species, for comparison.

Determination of the frequency of mutations and the spectrum of chromosome aberrations was carried out using meristematic cells of the vegetative cones. They were collected together with root tips from seeds of wheat and barley and fixed using the techniques of Pausheva (2). Seeds were collected at the end of July at different distances from the plant and were grown in the laboratory. Their root tips were cut off and fixed.

The testing of gaseous fluorides for their mutagenic activity was made by fumigation of barley seedlings with 0.02 to 0.2 mg HF/m^3 for 1 hour daily for 10 days in polyethylene chambers (0.15 m^3). After harvesting, the shoot tips (apical cones) of seedlings were cut off and fixed. The seedlings had no visible injuries. This method of seedling fumigation may be used for the testing of cytogenetic effects of various atmospheric pollutants and barley seedlings may be used as sensitive and effective cytogenic monitors.

The anaphase-test was used. More than 1000 anaphases were studied for each variant. The genetic materials were fixed in acetic acid-alcohol 1:3, and then were colored by Fëlgen (2). The samples were analyzed for fluoride using a fluoride selective ion electrode (3).

Results and Discussion

The fluoride levels found in the plants at each sample site in the study and control areas and the percentage of chromosome aberrations in root tips and shoot tips of grain crops are given in Table 1. It was established that the background mutation rate of plants growing in non-polluted areas was relatively low, but some mutations occurred mainly due to the use of fertilizers and pesticides.

A significant increase in the rate of chromosome aberrations in root tips of wheat and barley were found in plants near the fluoride source. The frequency of mutations in root tips from plants in this area was 2-6 times higher than in the control. The spectrum of chromosome aberrations was also changed to a great extent. Thus, in zones of chronic pollution with fluorine containing emissions from industry the percentage of the complex types of aberrations (chromosomal bridge, chromatic bridge, bridge with fragments, etc.) was increased and the amount of the simple ones (single fragments, twin fragments, etc.) was decreased (Figure 1). The relationship between the percentage of fragments and the percentage bridges in root tips of plants was 10:5 in control areas and it was 10:11 in polluted areas. The microphotographs of the main types of mutation are illustrated in Figure 2 and Figure 3.

It was established that the frequency of chromosome aberrations in shoot tips (apical cones) of wheat and barley of the polluted populations was 6.7 and 4.9 times as high as the control level, respectively (Table 1). The rate of mutation in wheat and barley was correlated with the distance the crops were located from the plant. The types of mutations in apical cones of the crops are illustrated in Figure 4.

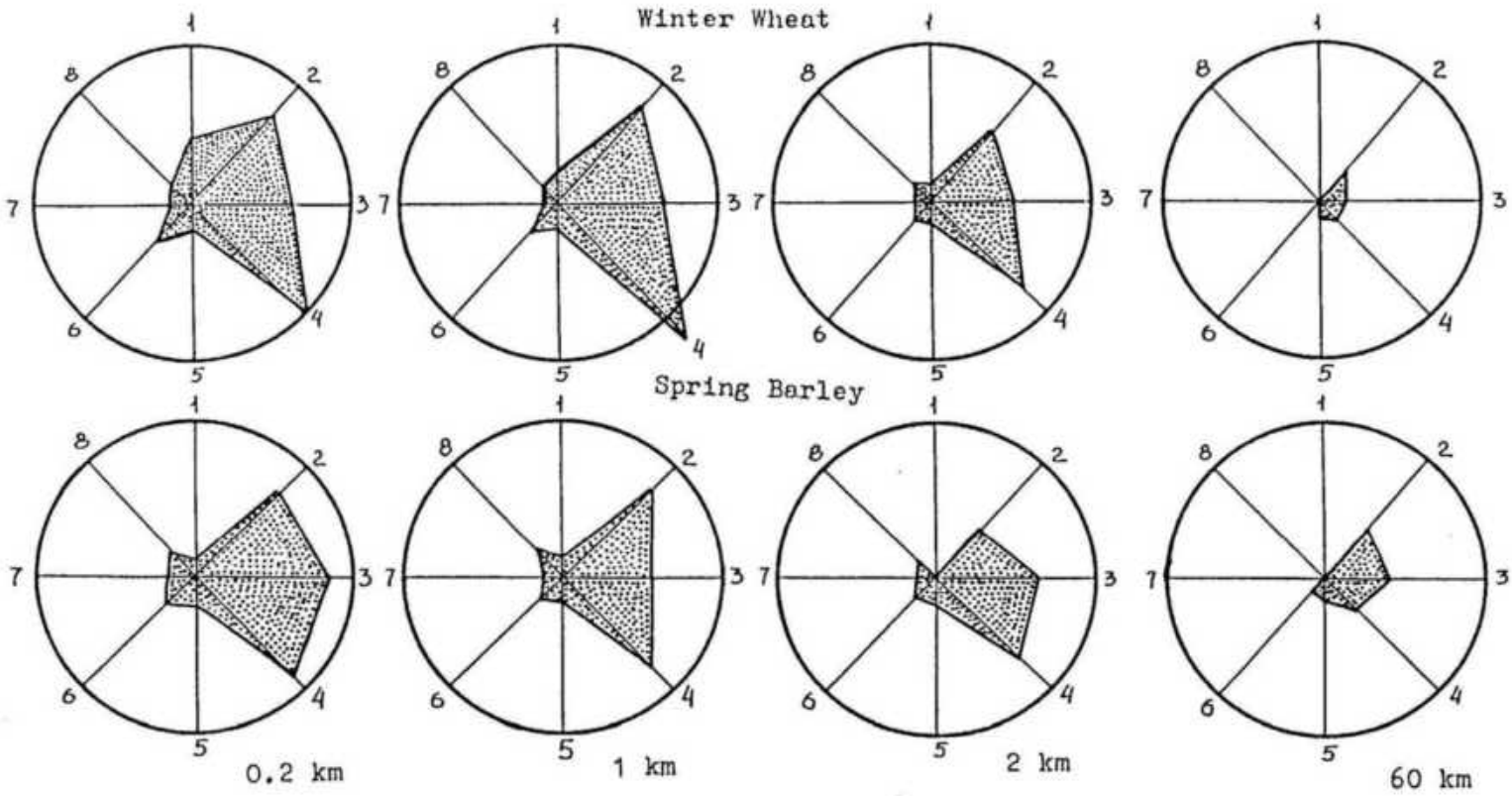
Although the highest fluoride content in the grain of wheat and barley was markedly less than that obtained from the green tissue of plants, the mutation rates in shoot tips and root tips of grain crops at similar locations were comparable. It may not be out of place to touch upon the problem of potential alterations (4,5). Results showed that grain crops, growing near the industrial plant, accumulated fluoride in high concentrations that were 5-120 times higher than in control areas (Table 1), which could lead to the beginning of potential alterations. Some of these alterations are present as chromosome aberrations in the vegetative cones, which are evident (Figures 1-4). The others remain until harvesting. These are the long-living potential alterations (4). It is probable that the potential alterations are induced by biochemical changes. The relationship between the chromosome aberrations and fluoride content in wheat and barley is shown in Figure 5.

TABLE 1

FLUORIDE ACCUMULATION IN PLANTS AND INDUCTION OF CHROMOSOME ABERRATIONS IN MERISTEMATIC CELLS FROM THE ROOT TIPS AND SHOOT TIPS OF GRAIN CROPS

Distance from plant	F^- accumulation (mg/kg dry weight)		No. of studied anaphases	Chromosomal aberrations	
(km)	Grain	Straw		Number	%
WINTER WHEAT	Root tips:-				
0.2	2.8 ±0.02	514.0 ±0.27	1003	134	13.4 ±0.93
1	1.9 ±0.02	79.0 ±0.76	1213	156	12.9 ±0.87
2	0.8 ±0.04	58.7 ±0.94	1144	86	7.5 ±0.95
60(control)	0.0	3.8 ±0.02	1097	24	2.2 ±0.13
	Shoot tips:-				
0.2		104.0 ±0.45	1001	127	12.7 ±0.81
1		14.1 ±0.28	1045	127	12.2 ±0.53
2		10.2 ±0.07	1204	100	8.3 ±0.12
60(control)		1.8 ±0.01	1050	20	1.9 ±0.10
SPRING BARLEY	Root tips:-				
0.2	6.2 ±0.03	276.0 ±0.14	1234	157	12.7 ±0.83
1	2.4 ±0.02	105.0 ±0.09	1117	105	9.4 ±0.91
2	1.3 ±0.02	87.2 ±0.13	962	76	7.9 ±0.54
60(control)	0.0	4.1 ±0.01	1084	40	3.7 ±0.25
	Shoot tips:-				
0.2		45.0 ±0.28	956	124	13.0 ±0.84
1		36.8 ±0.09	1078	97	9.0 ±0.92
2		19.9 ±0.14	1015	69	6.8 ±0.23
60(control)		0.8 ±0.01	1008	27	2.7 ±0.08

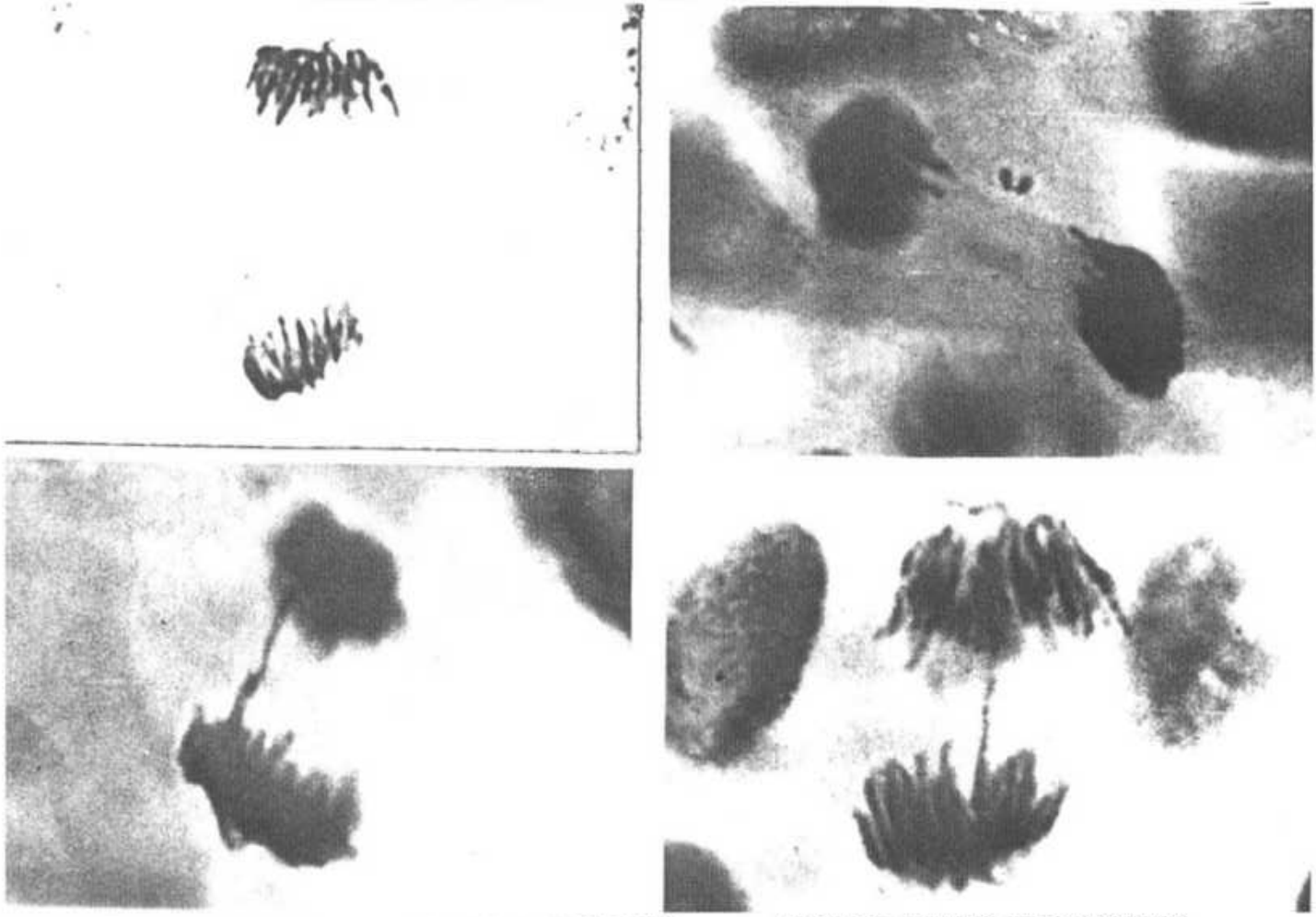
FIGURE 1. SPECTRUM OF CHROMOSOME ABERRATIONS IN ROOT TIPS OF GRAIN CROPS



RADIUS IS 4%. ZERO IS THE CENTRE OF THE CIRCLE.

1 - TWIN FRAGMENTS 2 - SINGLE FRAGMENT 3 - PLURAL FRAGMENTS 4 - CHROMATID BRIDGE 5 - CHROMOSOMAL BRIDGE
6 - SEVERAL BRIDGES IN THE CELL 7 - BRIDGE(S) WITH FRAGMENT(S) 8 - OTHER ABERRATIONS

FIGURE 2. SOME TYPES OF CHROMOSOME ABERRATIONS

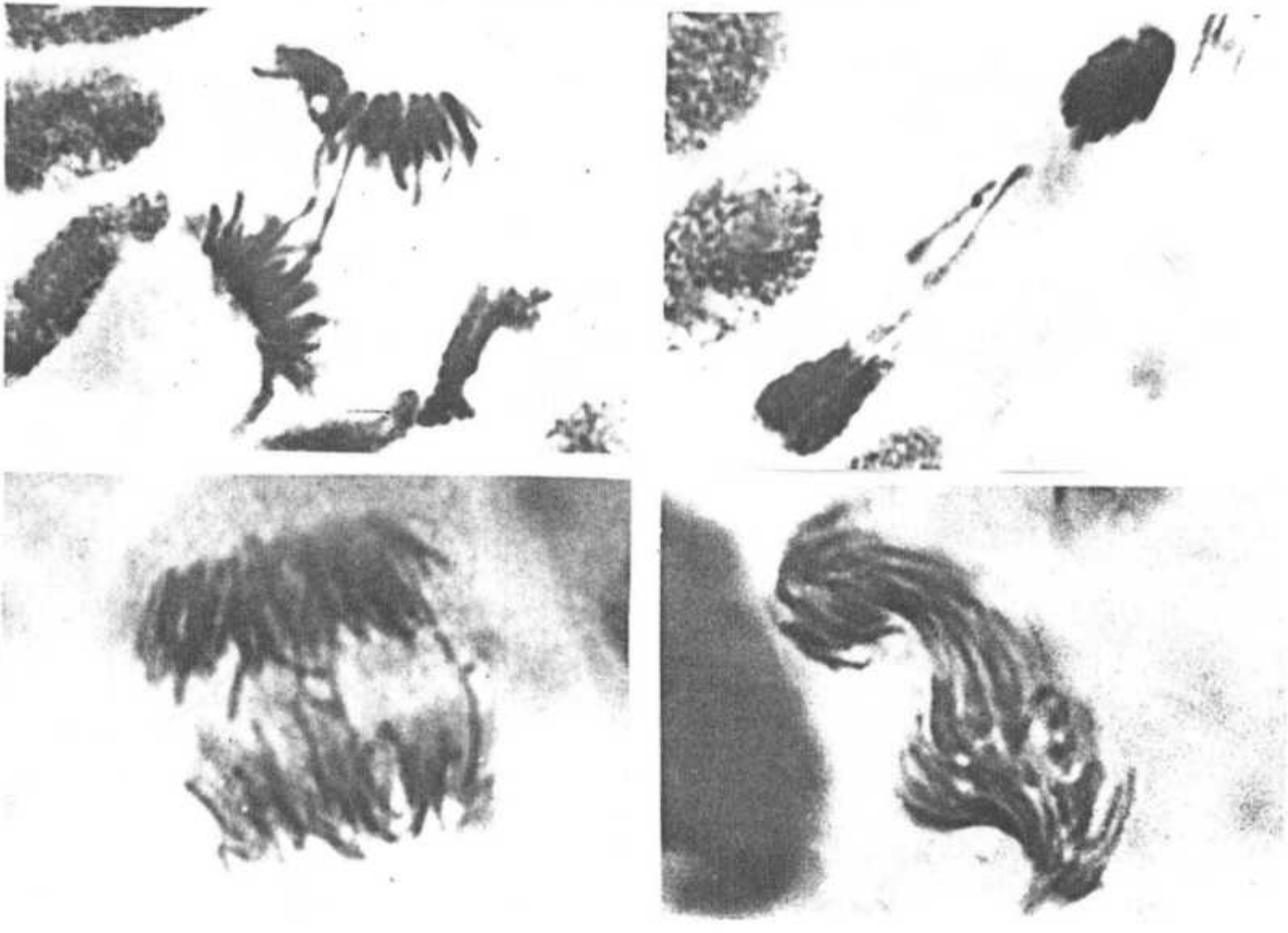


TOP LEFT: NORMAL ANAPHASE

TOP RIGHT: TWIN FRAGMENTS

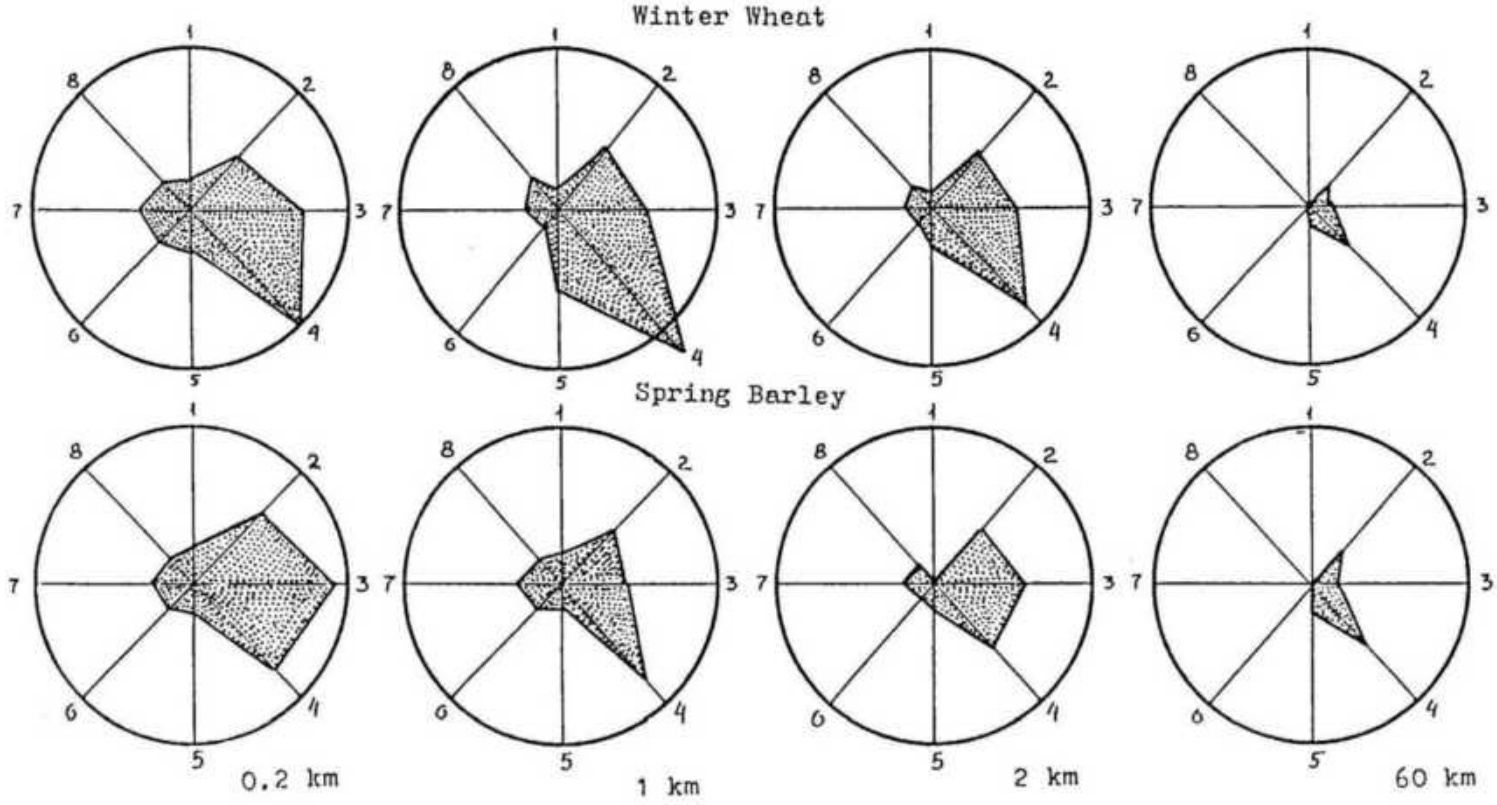
LOWER LEFT AND RIGHT: CHROMATID BRIDGES

FIGURE 3. SOME TYPES OF CHROMOSOME ABERRATIONS



TOP LEFT: CHROMOSOMAL BRIDGE TOP RIGHT: BACKWARD CHROMOSOME LOWER LEFT: SEVERAL ABERRATIONS LOWER RIGHT: OTHER ABERRATIONS

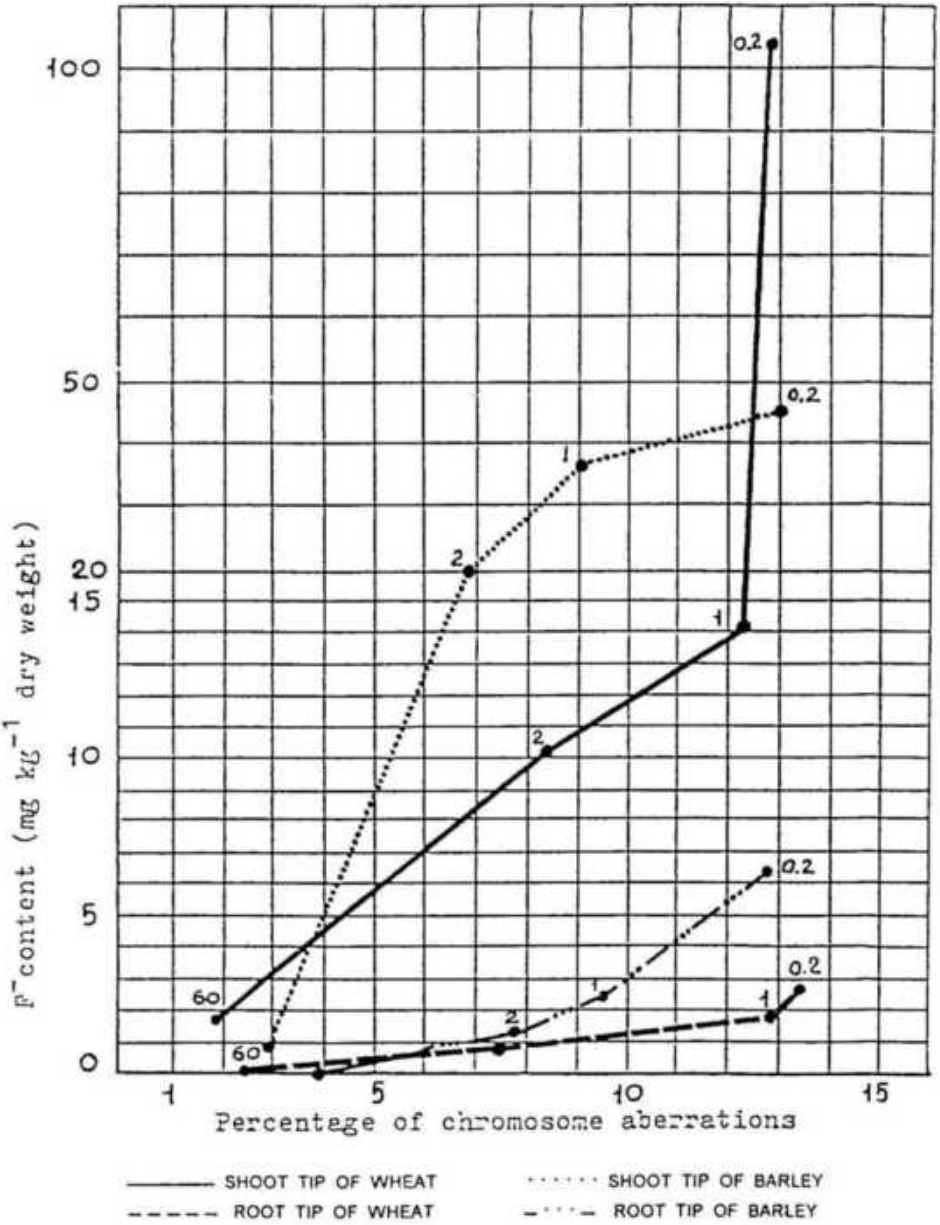
FIGURE 4. SPECTRUM OF CHROMOSOME ABERRATIONS IN SHOOT TIPS OF GRAIN CROPS



RADIUS IS 4%. ZERO IS THE CENTRE OF THE CIRCLE.

1 - TWIN FRAGMENTS 2 - SINGLE FRAGMENT 3 - PLURAL FRAGMENTS 4 - CHROMATID BRIDGE 5 - CHROMOSOMAL BRIDGE
6 - SEVERAL BRIDGES IN THE CELL 7 - BRIDGE(S) WITH FRAGMENT(S) 8 - OTHER ABERRATIONS

FIGURE 5.
RELATIONSHIP BETWEEN THE CHROMOSOME ABERRATIONS AND FLUORIDE CONTENT IN PLANTS

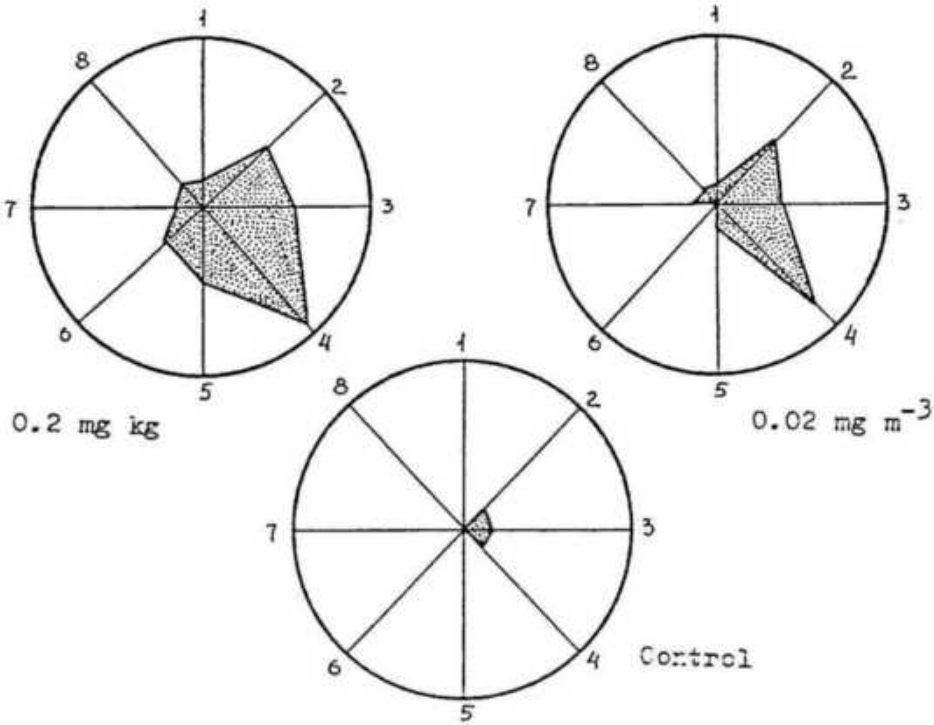


THE FIGURES 0.2, 1, 2, AND 60 ARE THE DISTANCE FROM THE PLANT IN KILOMOTERS

TABLE 2
FLUORIDE ACCUMULATION AND CYTOGENETIC EFFECT OF HF FUMIGATION ON BARLEY SEEDLINGS

HF concentration ($mg\ m^{-3}$)	F^- accumulation (mg/kg^{-1} dry weight)	No. of studied anaphases	Chromosomal aberrations	
			Number	%
0.2	25.3 ±1.11	1086	122	11.2 ±0.76
0.02	2.7 ±0.08	1115	58	5.2 ±0.08
Control	0.0	1112	13	1.2 ±0.03

FIGURE 6. SPECTRUM OF CHROMOSOME ABERRATIONS IN VEGETATIVE CONES OF BARLEY SEEDLINGS



RADIUS IS 4%. ZERO IS THE CENTRE OF THE CIRCLE.
1 - TWIN FRAGMENTS 2 - SINGLE FRAGMENT 3 - PLURAL FRAGMENTS
4 - CHROMATID BRIDGE 5 - CHROMOSOMAL BRIDGE 6 - SEVERAL BRIDGES IN THE CELL
7 - BRIDGE(S) WITH FRAGMENT(S) 8 - OTHER ABERRATIONS

Our calculations have indicated that the speed of the mutations in the meristems of winter wheat was on average 2.3 times lower than in spring barley. It should be emphasized that winter wheat has a 7-month growing season (without 2 winter months) whereas spring barley has a 3-month season. The percentage of chromosome aberrations in the wheat, however, is similar to that found in barley. Winter wheat is much more resistant than spring barley due to the hexaploidy of its genome (wheat has 42 chromosomes and barley has 14 chromosomes). It is evident that fluoride mutagenicity depends largely upon the plant species.

Results of laboratory experiments testing HF for its mutagenic activity by fumigation of barley seedlings in chambers are given in Table 2. The percentage of chromosome aberrations in apical cones of barley seedlings for the 0.2 mg/m^3 HF-treated groups was 9.3 times higher than in the control. HF fumigation induced not only a high mutation rate, but also alterations in the spectrum of chromosome aberrations (Figure 6). The HF-induced mutation rate was correlated linearly with dose of pollutant.

Barley seedlings are highly sensitive to mutagens in gaseous forms such as HF and may be used for the screening of mutagens and as cytogenetic monitors for chemical agents. Thus, gaseous fluorides (HF) resulting from industrial emissions are highly mutagenic for grain crops.

Plant responses to the widespread atmospheric pollutant, fluoride, have been documented in detail (6-9), but the mechanism of the mutations induced by HF is unknown. Several possibilities based on previous studies of researchers suggested the mechanism of HF mutagenicity was at the biochemical level, but further experimental studies are needed to elucidate the mechanisms involved.

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