THE FIRST DETECTED CASE OF AMITOSIS IN PINE

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ABSTRACT

The first example of amitosis in a progeny of a pine *(Pinus sylvestris* L.) is presented, from a tree growing near the Novozybkovsk highway in the Bryansk region of Russia. The area was contaminated by radionuclides as a consequence of the Chernobyl accident, but to a lesser extent than other regions of Bryansk. Replacement of normal mitosis by amitosis in this case apparently was caused by the synergic effect of low doses of radiation and chemical pollution from the exhausted gases of motor transport.

Key words: *Pinus sylvestris* L., amitosis, the Chernobyl accident, Bryansk region, chemical pollution

INTRODUCTION

The Bryansk region is a region of Russia that was affected by radiation as a consequence of the Chernobyl accident. The southwestern part of the Bryansk region suffered to the greatest degree from the irradiation. These areas are mainly forests with stands of pine, oak and sitka spruce. ^{135}Cs, which has a long half-life, was a major component in the gamma-background in contaminated stands, whereas 15 % was middle half-life ^{134}Cs and only 5% was short half-life ^{144}Cs and ^{166}Ru. Forests were the barriers that delayed the spread of radionuclides after the nuclear accident. The radionuclides were concentrated mainly in the tree crowns, but during the next two years they were transferred to the litter and partially incorporated into trees through the root system, causing both external and internal irradiation of the trees.

Since 1983, scientists of the Bryansk Technological Institute have examined the level of gamma background in contaminated areas. They have found that the level of contamination is falling in all areas. After 1990, it decreased to about 20 % of the initial level. Some test areas where gamma background varied from 0.01 to 1.3 mR/h were selected for analysis. Effects of the chronic influence of low doses of radiation on tree growth, development and sexual reproduction were studied. *Pinus sylvestris* L. was monitored because this species is widespread in stands of the Bryansk region and its radiosensitivity is similar to that of humans. The trees examined were approximately 65 years old, growing in high-productive stands.

Cytogenetic monitoring of trees from the test areas with gamma background levels of 0.05, 0.52, 0.92, and 1.3 $mR.h^{-1}$ was carried out from 1991 to 1993. A family of four trees was used as control. These four trees grow in the Study Trial Forestry Nursery of the Bryansk Technological Institute, where gamma background is 0.01 $mR.h^{-1}$. One of the control trees grows near the Novozybkovsk highway. Seed samples were collected in autumn.

MATERIALS AND METHODS

The fresh seeds were germinated in Petri dishes on moist filter paper. The germination rate was 84–90 % in all samples. For the cytological investigation, plantlets with roots 0.5–1.0 cm long were fixed in Carnoy's mixture (3 : 1, ethanol : glacial acetic acid). All materials were stained with aceto-haematoxylin. Permanent slides were prepared by squashing pre-stained root tips in a drop of Houer's mixture. An MBI–15 microscope was used for slide examination.

RESULTS

Cytological investigation of the progeny of experimental and control groups of trees during 1991–1993 showed that as gamma background decreased, cell processes returned to normal. The frequency of cytological abnormalities in these plantlets varied from 1.5 to 3.5 %, but no correlation with contamination level was observed. However, mitotic activity in seeds from the more contaminated areas was lower (7–7.9 %) than it was in seeds from the less contaminated areas (8.3–9.0

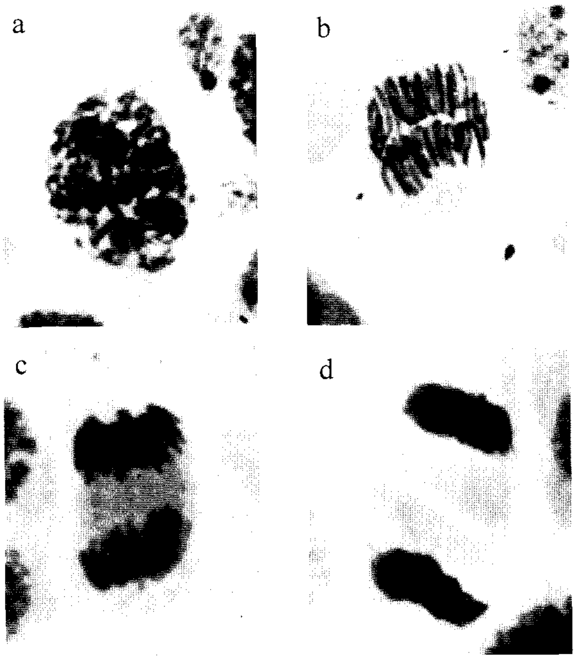

Figure 1 Normal mitosis in pine: a – prophase, b – metaphase; c – anaphase, d – telophase

%). Bridges, premature movement of several chromosomes to metaphase poles and delay of chromosomes in anaphase were the prevalent types of cytological abnormalities in all samples.

One of the control trees growing near the Novozybkovsk highway differed from the others by the cytological characteristics of its progeny, although the tree itself was phenotypically similar to the others. The progeny of this tree had a high frequency of cytological abnormalities (c. 7 %). The specific type of cytological abnormality was identified as the occurrence of cells with micronuclei. Their frequency was approximately 5 % of the total number of interphase cells. Cells were also found in which normal mitosis was replaced by amitosis. Figure 1 shows all typical stages of mitosis. Figure 2 demonstrates bridges with (a) and without (b) fragments in anaphase. Some cells with micronuclei are shown in Figure 3b. Micronuclei are usually formed as a result of disturbance in chromosome movement when particular chromosomes are not included in a daughter nuclei and are isolated as independent micronuclei (Figure 3a, b). However, some micronuclei may originate from amitosis. In such a case, the maternal nucleus has an irregular shape. Such a nucleus may segregate near the nuclear membrane, as in Figure 3c; part of it then becomes surrounded by cytoplasm and separates from the main body.

Amitosis as a means of micronuclei formation was described earlier by PROKOFIEVA–BELGOVSKAYA (1953) in potato. Sometimes, binucleate cells may arise by amitosis (Figure 3c–3e). In this situation, the nuclear

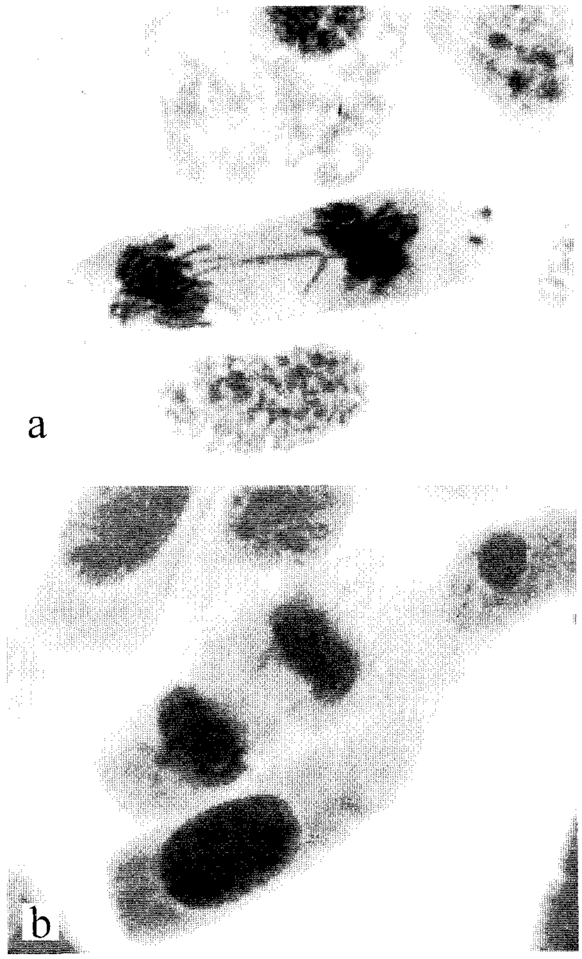

Figure 2 Chromosome bridges with (a) and without (b) fragments in anaphase of the pine tree growing near the Novozybkovsk highway (Bryansk region, Russia)

plate usually divides the maternal nucleus into two unequal parts and both of the daughter nuclei remain close together. An interesting observation was the occurrence of amitosis in one of the daughter cells derived from mitosis of a mother cell with incomplete cytokinesis (Figure 3c).

DISCUSSION

Amitosis as a direct method of nuclear division is characteristic of cells of degenerating tissues and can be induced by chemical or physical factors inhibiting mitosis (URYVAEVA 1965; JINKIN 1966).

In 1986, we analyzed cytologically seedlings from acorns collected from oak trees growing in a 30 km zone around the Chernobyl Nuclear Power Plant. We found many different abnormalities in their meristematic cells, and replacement of mitosis by amitosis was conditions of strong radioactive pollution after the Chernobyl accident, which happened at the time when he most frequent type. These acorns were formed in

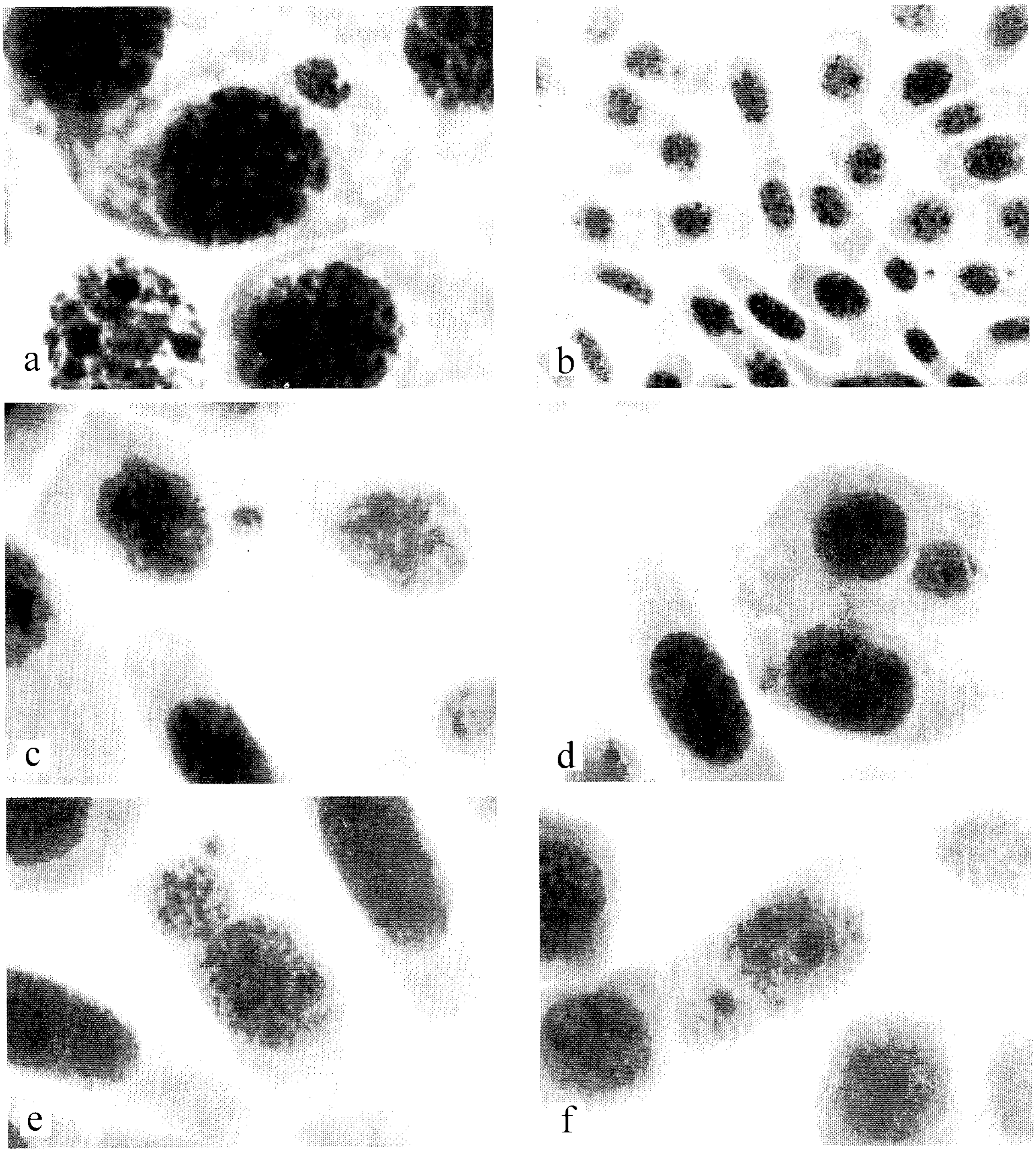

Figure 3 Different ways of micronuclei formation in the pine tree growing near the Novozybkovsk highway, Bryansk region, Russia. **a – b** cells where micronuclei were formed as a consequence of disturbance of chromosome movement, when several chromosomes are not included in a daughter nuclei and are isolated as an independent micronuclei; **c – f** cells with micronuclei originated from amitosis

meiosis in oak, pine, and many other plants took place. Cells with this type of abnormalities were eliminated during subsequent years.

The phenomenon of amitosis observed in meristematic tissues of a seedling progeny of the pine tree growing under low-radiation conditions (gamma-background 0.01 mR.h^{-1}) may be explained by the synergic effect of low doses of irradiation and chemical mutagenes from the exhausted gases of automobiles along the highly travelled route from the zone of the Chernobyl Nuclear Power Plant. Such an effect is suggested by our data. This synergism might have as much effect on organisms as a higher level of chronic radiation has without it; it would appear that this should be kept in mind. It is recommended that gathering mashrooms, berries, and medical herbs along the highways should be avoided, as should the pasturing of cattle in these areas. Protective reforestation should be created in front of houses near highways.

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