Abstract. The aim was to evidence the response of 5-day-old maize seedlings to X-ray doses up to 200cGy, by studying plant growth and development, biosynthesis of chlorophylls and chromosomal aberrations. The plant response after exposure to ionizing radiation shows slight inhibition of biosynthesis of pigments for lowest dose radiation with a tendency of stimulation for higher doses. For two step irradiation - first a low dose of 50cGy then, after 10 minutes, a higher dose of 200cGy, both pigment biosynthesis and the accumulation of green substance were stimulated, which can be explained by the adaptive response of living organisms to low doses of radiation. Chromosomal aberrations such as interchromatidian bridges, lagging and expelled chromosomes and multipolar divisions were evidenced. The mitotic activity was found to be a cytogenetic parameter quite sensitive to low level irradiation with X-rays as being slightly increased for 50 cGy and 75 cGy doses.

Key words: low dose X-rays, photoassimilatory pigments, aberrant division

1. INTRODUCTION

The first experiments on caryopses exposed to relatively low doses of ionizing radiation in order to stimulate growth and development were performed a few years after the discovery of X-rays by Roentgen in 1895. Such exposure stimulated seed germination, plantlet growth, flowering, plant size and yield as reviewed by Breslavets (1946) [1]. These different results allowed Patskevich (1961) [2] to conclude that irradiation of seeds prior to sowing may represent a great promise from the viewpoint of its practical application in agriculture. It was generally agreed that low doses of gamma rays stimulate cell division, growth and development in various organisms not only plants but also animal organisms. This phenomenon, named “hormesis”, has been analyzed and discussed by several authors related to different plant species (Luckey, 1980[3]; Sagan, 1987[4]; Planel et al., 1987[5]; Korystov and Narimanov, 1997[6]).

However, the mechanisms explaining radiation influence on plant growth and development are still insufficiently described so that the available data remain controversial. Indeed, the magnitude of the reported hormeric effects of radiation is usually small, being of approximately 10% of control values and often not providing critical evidence that crop yield are significantly increased by irradiating seeds (Miller, 1987)[7]. For example, an inhibition of pea seedling growth was obtained after gamma irradiation ranging from 15 to 100 Gy (Bagi et al., 1988) [8]. Croci et al. (1991) [9] evidenced that gamma irradiation (10 Gy) of post-dormant garlic cloves induced
significant inhibition of sprout growth. Also, doses between 0.5 and 20 Gy were found able to induce morphological and cytological changes (micronuclei, chromosomal aberrations and reduction of mitotic index) in onions (Vaijapurkar et al., 2001) [10] while beta irradiation with doses ranging between 0.2 and 1.2 Gy did not produced significant biochemical changes although stimulatory influence on the growth of plants was revealed by Chicea et al. (2006, 2008)[11-12].

The effects of ionizing radiation on DNA produce noticeable chromosomal aberrations during either mitosis or meiosis. The most frequent aberrations seen during meiosis are desynapsis, abnormal anaphase disjunction, multipolar separation or the formation of tetravalent or even hexavalent chromosomes in species that normally have ring-shaped divalents. Consequently, chromosome malsegregation and defects in chromatid separation, bridge formation, chromosome exchange, chromosome breakage or loss of chromosome fragments were reported (Chenal et al., 2006 [13]; Geraskin et al., 2005 [14]; Misra et al., 1991 [15]; Shirley et al., 1992 [16]). These numerous abnormalities generate acentric fragments that are excluded during the late-stages of mitosis and produce micronuclei that reflect also the result of these aberrations (Zaka et al., 2002a [17]). The number of micronuclei increases in a dose-dependent manner in irradiated root-tip cells of garlic (0.06–0.26 Gy) and peas (up to 10 Gy) (Cortes et al., 1990 [18]; Zaka et al., 2004 [19]). In peas, the dose–effect relationship is supra linear with a specific effect at low doses (0.4 Gy) (Zaka et al., 2002a, 2004). Ionizing radiation in Arabidopsis resulted in sequence inversions and deletions (Singh, 1974 [20]) as well as in chromosome homologous exchanges, which were found to be dose dependent above 5 Gy (Kovalchuk et al., 2000a [21]).

With the rising issue of environmental radioactive pollution, involving relatively low radiation doses within contaminated areas, it is necessary to collect more and more reliable data on the effects of such radiations on biological organisms.

2. MATERIAL AND METHODS

Maize caryopses with uniform genophond – provided by the same plant, chosen for its biological superior features were let to germinate on watered porous paper support in Petri dishes (100 seeds each) in darkness, at about 24 °C temperature. The second day after irradiation root meristem aliquots were taken for cytogenetic investigation that was based on microscopic observations and cell counting. At the seedling age of 14 days the spectrophotometric analyses and growth parameter assessing were performed.

2.1 X-ray Exposure

The freshly germinated caryposes were irradiated using a kilovoltage X-ray tube (SRT100 characterized by 70 kV and 10 mA; the filtration system being based on
0.75 mm Al and HVL: 1.3 mm Al.) - a superficial radiotherapy device used in the "St. Spiridon" Emergency County Hospital, Iasi, Romania. The Petri dishes containing the vegetal samples were exposed to the dose rate of 227cGy/min at a source-dish distance of 25 cm. The time of exposure was calculated at the level of the seedlings, using classical computerized software of radiotherapy treatment planning system.

The literature being limited regarding the effects of low doses on plants, we focused on a range of doses below 5 Gy. For this purpose, we irradiated the seedlings with five small doses: 50; 75; 100; 200 cGy for unique irradiation and 50+200cGy for the two step irradiation. These doses may be taken as very small (50cGy) or relatively small doses (100 and 200 cGy) considering the usual high radio resistance of plants.

2.2 Pigment Assay and Growth Assessing

The levels of chlorophyll a, chlorophyll b and total carotenes from the green tissues were assayed in the pigment acetone (85 %) extract according to Meyer Bertenrath method modified by Stirban and Farcas [22]. Shimadzu UV-170 spectrophotometer with 1 cm width quartz cells was used for light absorbance measurement in the acetone extract spectra. The calculation formulae were those of Lichtenhater [23]. Statistic analysis by means of t-test was carried out considering the significance threshold of 0.05. Plant growth at the 14th day was evaluated by means of fresh substance mass (using semi-analytical balance with 10^-4 g accuracy) and plant height (usual ruler with 1 mm accuracy).

2.3 Cytogenetic Investigation

For selective staining of nuclear material - the chromosomes, we used the Feulgen method [24]. The method is based on the fact that, selectively, only chromatin chromosomes stained reddish-purple with alkaline fuxin, while RNA in nucleoli and cytoplasm and other cellular components remain not stained. The final samples were obtained by squash method, the meristeme tissue being arranged on glass slides. Nikon microscope with 400x magnitude was used to analyze the cells.

Quantitative cytogenetic analysis was based on counting cells frozen in various stages of mitotic division - either normal or aberrant divisions - compared with those in interphase and chromosomal aberrations assessing (abnormal mitotic divisions). Chromosomal aberrations such as interchromatidian bridges – either singular or multiple bridges, lagging and expelled chromosomes as well as multipolar divisions were evidenced.

Mitotic index (MI%) and chromosomal aberrations occurrence index (A.I.%), indicates the percentage of chromosomal aberrations induced by irradiation:

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MI(\%) = \frac{\text{total.dividing.cells}}{\text{total.analyzed.cells}} \times 100
\] (1)
\[ AI(\%) = \frac{\text{total.chromosomal.aberrations}}{\text{total.analyzed.cells}} \times 100 \] (2)

Comparative discussion regarding the two types of irradiation by means of graphical plots was accomplished.

3. RESULTS AND DISCUSSION

The average values of photosynthesis pigment resulted from three repeated measurements carried out in the same conditions are presented in the next graphs. As shown in Figure 1 the chlorophyll \(a\) concentration was found significantly increased in the exposed samples compared to the control non-exposed ones – with up to 100% for 50 cGy. The highest increase – with 120% was noticed in the two step irradiated sample (50+200 cGy). The same can be seen in the case of chlorophyll \(b\) – with still higher increase (with up to 165%) for the 50 cGy compared to the control. The total carotene concentration is presented – showing the same general tendency of variation (increase for unique irradiation dose as well as for the two step irradiation dose of 50+200 cGy).

![Figure 1 - The effect of X-ray low doses on chlorophyll \(a\), chlorophyll \(b\) and total carotenes in maize (standard deviation – 6\%)](image)
The chlorophyll ratio was significantly diminished, with about 30% for the lowest dose, of 50 cGy (Figure 2) as consequence of the fact that *chlorophyll a* increase was not so high as that of the *chlorophyll b*; non-significant changes were noticed for higher doses except the dose of 200 cGy where an increase was noticed (of about 20% with statistic significance – \( p<0.05 \)). This ratio of chlorophyll concentrations that gives an indirect measure of photosynthesis efficiency showed that photosynthesis is generally a radioresistant process; however in the case of the lowest dose applied within this experiment a special sensitivity to radiation exposure was revealed.
The fresh substance mass (Figure 3) presented slight but significant increase (p<0.05) for 50 cGy and 50+200 cGy – the other variations having no statistical significance while plant average height (averaging for all plantlets within a Petri dish) is generally higher for all applied doses compared to the non-irradiated control (p<0.05) - both biological growth parameters indicating slight stimulatory effect of X-ray low doses on the metabolism of maize seedlings during early ontogenetic stages.

From both photoassimilatory pigment graphs and those of the growth biological parameters it seems that the effects of the two step irradiation applied in this experiment needs special attention due to the stimulatory effects evidenced in all above figures; one can assume that the interval between the two doses application favored the mobilization of the defense cellular mechanisms so that the molecular damages of first irradiation step were repaired, their compensation resulting even in slight (but significant) stimulatory effect – following the second irradiation step.

The Figures 5 present the results obtained by the cytogenetic investigation of irradiated samples compared with non irradiated ones. From more than 30,000 cells taken into account the main types of chromosomal aberrations that could be identified were: the interchromatidian bridges (either singular or multiple) – the two sets of chromosome resulted after DNA duplication being unable to separate so that a diploid cell resulted instead of two normal cells, the multipolar divisions – the chromosomes being grouped in more than two nuclei, as well as the lagging and expelled chromosomes in anaphase, metaphase and ana-telophase – meaning that parts of genetic information bear on those chromosomes is lost (Figure 5 a, b).
Figure 5a - The frequency of interchromatidian bridges, lagging and expelled chromosomes in anaphase identified in irradiated samples

Figure 5b - The frequency of multipolar anaphases, metaphases with expelled chromosomes and C-metaphases identified in irradiated samples
The cytogenetic tests were performed by microscopic observation of 10 fields for each sample corresponding to each irradiation dose. Standard deviation was taken as error bar in each case. Student \( t \)-test was applied to evidence statistical significance according to the threshold of 0.05.

The results show that the number of most types of chromosomal aberrations has a tendency to increase with increasing the absorbed dose. For example, in the samples irradiated with 200 cGy were identified five times more simple bridges, compared with non irradiated ones; also the number of multiple bridges increased approximately five times after the longest irradiation time (Figure 5a). After 75 cGy, the c-metaphases began appearing, reaching a rate of 1.62% of the total cells captured in various stages of division, for the samples exposed with the highest dose (Figure 5b).

Chromosomal fragments which remained in the space between the two sister cells, will change the genetic information to daughter cells resulting from division. Because of chromatide bridges results in these experiments, the cells will exhibit an incomplete chromosomal set.

In Figure 5c an example presenting the chromosomal aberrations evidenced during the cytogenetic analysis is given.

![Figure 5 c - Normal anaphase (left); anaphase with lagged chromosome (1) and expelled chromosome (2) (right)](image)

The mitotic index (M.I.) showed an increasing trend for the lower-doses of X-ray radiation (50 cGy and 75 cGy) with a maximum of 7.54% corresponding to 100 cGy dose – which suggested the possible stimulating effect of low dose radiation on the cell mitotic activity. A progressive decreasing of M.I. – up to a value of 6.1 % in the samples exposed to 250 cGy was also evidenced (Figure 6); this level is practically the same as for the control samples.
The chromosomal aberration index (A.I.), generally increase with the increase of the exposure time, from 0.58% (corresponding to control samples) to 5.84% for the highest irradiation dose.

It seems that repair mechanisms could not balance the disturbing effect induced by radiation absorption in the DNA molecules of the cell nucleus. Possibly some of such aberrant divisions propagate as genetic mutations in some cases.

![Figure 6 - Mitotic index and chromosomal aberration index vs. X-ray dose](image)

4. CONCLUSION

Since anthropogenic civil and military nuclear activities can contribute to accidental short-term exposures in addition to the chronical natural exposure due to both telluric and cosmic radiation, it is necessary to develop studies on inheritance of the effects of low and very low doses on plants as well as on animals in order to obtain models potentially useful in conservation biology and radio-protection for humans.

The low doses of X-ray energy (50-75-100 cGy) had induced slight stimulatory effect in the young maize plantlets, at the level of photosynthesis pigment production; however the photosynthesis efficiency was not significantly changed and neither the accumulation of biomass. For the dose of 200 cGy as well as for 50+200 cGy – which required longer irradiation time, the accommodation of the young plant organism could occur so that the disturbances induced at the beginning were repaired latter and even stimulatory influence on the plant metabolism has resulted.

Both fresh substance mass and plantlet height indicate the slight stimulation of metabolism – induced by low radiation doses of the order of magnitude of 200 cGy. It
can be assumed that the two step irradiation has mobilized the defense cellular mechanisms so that the damages of first irradiation step were repaired, their compensation resulted even in certain stimulatory effect – following the second irradiation step.

The genotoxicity of low X-ray doses on maize root tissue was proven, because the percentage of chromosomal aberrations increased very much, up to ten times, compared to the control samples. The mitotic activity was found to be a cellular process sensitive to low level irradiation with X-rays since the mitotic index was slightly increased for 50 cGy and 75 cGy doses.

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REFERENCES