

Aberrant cell divisions in root meristeme of maize following exposure to X-rays low doses compared to similar effects of 50 Hz electromagnetic exposure

R. FOCEA^{1*}, G. CAPRARU³, M. RACUCIU², D. CREANGA¹, T. LUCHIAN¹

¹ “Alexandru Ioan Cuza” University, Faculty of Physics - Iasi, Romania

² “L. Blaga” University, Faculty of Sciences - Sibiu, Romania

³ Institute of Biological Research - Iasi, Romania

Abstract

The response of maize to radiation exposure was investigated by two cytogenetic methods considering the importance of the genotoxic effect for environmental and agricultural purposes. Uniform genophond seeds, freshly germinated, were exposed to relatively low radiation doses using a radiotherapy X-ray applicator from a hospital irradiation device and to a 50 Hz electromagnetic field with about 10 mT magnetic induction (generated within laboratory assembled electromagnetic coils). Radicular meristeme tissue aliquots were prevailed for cytogenetic investigation based on microscopic observations and cell counting. Microscope slides were prepared following a specific procedure (squash technique and Feulgen method based on modified Carr reactive coloration). Mitotic index as well as chromosomal aberration percentage were calculated for more than 30,000 cells taken into account. From a qualitative viewpoint, chromosomal aberrations such as interchromatidian bridges, lagging and expelled chromosomes and multipolar divisions were evidenced – no distinct situation for either ionizing radiation or electromagnetic field being identified. The main

*E-mail: ramona_focea@yahoo.com

quantitative difference consisted in the increased mitotic index for electromagnetic exposure increased times compared with the diminished mitotic index in the case of low X-ray doses.

1. Introduction

Over the past decades some pioneer scientists reported that low-dose ionizing radiation is not only a harmless agent but often has a beneficial or hormetic effect. That is, low-level ionizing radiation may be essential trace energy for life, analogous to essential trace elements. Despite the fact that high doses of ionizing radiation are detrimental, substantial data from both humans and experimental animals show that biologic functions are stimulated by low dose radiation. In order to evidence the effects of gamma rays on mitotic division and plant growth many studies were done with different plant species. SavaÖkan and Toker [1] found that 50, 100, 150 and 200 Gy doses of gamma rays decreased plant size as well as mitotic index on *Secale cereale*. Al-Salhi *et al.* [3] studied the effects of gamma-irradiation on the biophysical and morphological properties of corn plants. Okamoto and Tatara [4] as well as Eroglu *et al.* [5] showed that cell division frequency was decreased by low-dose gamma irradiation on barley. Physiological and biochemical processes in plants are significantly affected by gamma-irradiation stress. The irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein [6], hormone balance, leaf gas-exchange, water exchange and enzyme activity [7].

2. Experimental

2.1. Biological material

Maize seeds 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. After germination, the exposure to X-rays as well as to an electromagnetic field was carried out. Root meristeme aliquots were taken for cytogenetic investigation based on microscopic observations and cell counting.

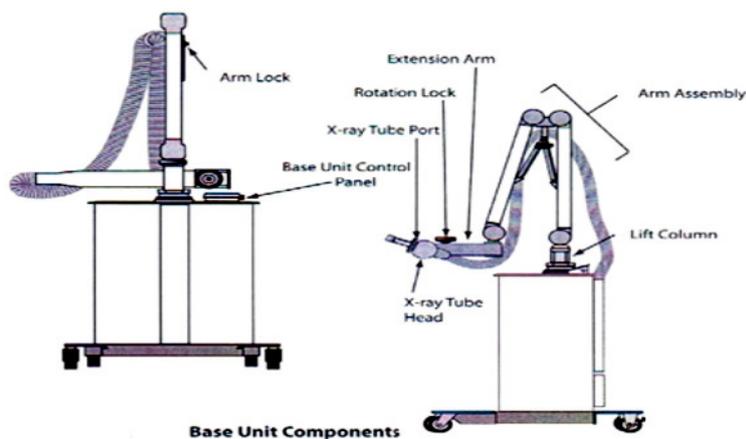


Fig. 1: The scheme of the TOPEX SRT 100 X-ray exposure system.

2.2. X-ray exposure

The TOPEX SRT 100 system (Fig. 1), dedicated to superficial radiation therapy was utilized; the X-ray generation source was characterized at 70 kV and 10 mA; the filtration system was based on 0.75 mm Al with an HVL of 1.3 mm Al.

The irradiation of freshly germinated seeds was carried out at the dose rate of 227 cGy min^{-1} at 25 cm (unique irradiation doses of 50; 75; 100; 200 cGy and the two step irradiation dose: 50+200 cGy being applied).

2.3. Electromagnetic exposure

The Helmholtz coil system assembled in laboratory was supplied from the national electricity network (50 Hz) with electric current able to generate a uniform magnetic field of 10 mT magnetic induction within a 10 cm diameter central zone. The maize seeds germinated in Petri dishes on watered porous paper were placed in the coil center. The exposed samples differ from each other by the exposure time duration: 1h; 2h; 4h and 6h. The non-exposed seeds were kept in the same environmental area (to avoid the putative influence of temperature or humidity gradients).

2.4. Cytogenetic investigation

For selective staining of nuclear material, the chromosomes, we used the Feulgen method. The method is based on the fact that, selectively, only chro-

matin chromosomes stained reddish-purple with alkaline Fuxin, while RNA in nucleoli and cytoplasm and other cellular components are not stained.

Quantitative cytogenetic analysis is 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 – no distinct situation for either ionizing radiation or electromagnetic field being identified.

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

$$(1) \quad M.I.(%) = \frac{\text{total diving cells}}{\text{total analyzed cells}} \times 100,$$

$$(2) \quad A.I.(%) = \frac{\text{total chromosomal aberrations}}{\text{total analyzed cells}} \times 100.$$

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

3. Results and discussion

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 multipolar divisions as well as the lagging and expelled chromosomes in anaphase, metaphase and ana-telophase (Figs. 2-3). No distinct situation for either ionizing radiation or electromagnetic field was revealed.

The following graphs present the results obtained by the cytogenetic investigation of irradiated samples compared with non irradiated ones, for both X-ray exposure and electromagnetic exposure.

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 the M.I. – down to a value of 6.1% in the samples exposed to 250 cGy – was also evidenced (Fig. 4); this level is practically the same as for the control samples.

The chromosomal aberration index (A.I.) generally increases with the increase of the exposure time, from 0.58% (corresponding to control samples)

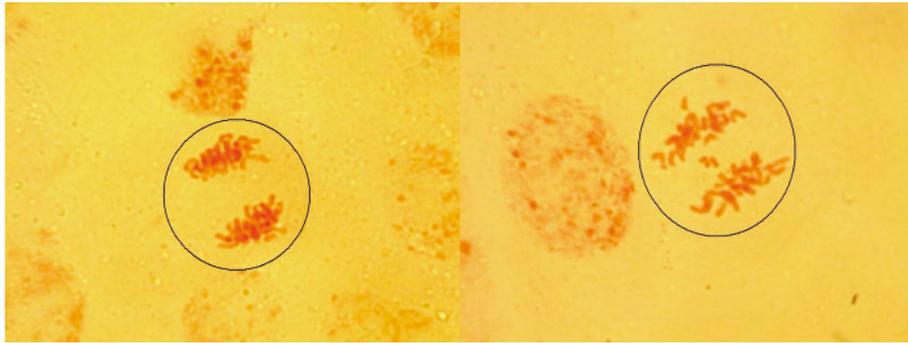


Fig. 2: Normal anaphase in maize root cells (left) and ana-telophase with lagging and expelled chromosome (right).

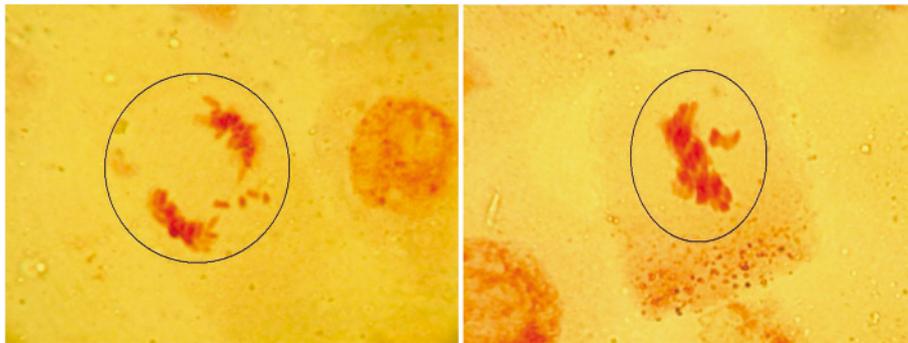


Fig. 3: Anaphase with lagging and expelled chromosomes (left) and metaphase with expelled chromosome in maize (right).

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.

A linear dose-response function could be proposed for the mathematical approach of chromosomal aberration percentage dependence on the radiation exposure level (proportional to the irradiation dose).

In the case of electromagnetic exposure, both the mitotic index and the occurrence of chromosomal aberrations index showed a tendency to increase as exposure time increased (Fig. 5). The M.I. continuously increased from about 3.6% to a more than twice higher value of about 9.1%, most probably due to stimulatory influence of electromagnetic energy absorption. A linear approach of the M.I. dependence on the electromagnetic time was evidenced

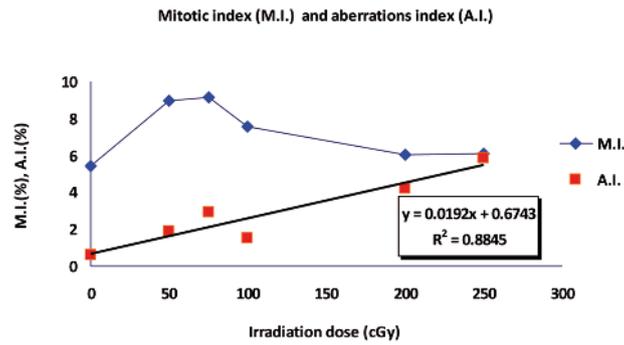


Fig. 4: Mitotic index and chromosomal aberration index *vs.* X-ray dose (y : A.I. (%); x : X-ray dose (cGy); R : linear correlation coefficient).

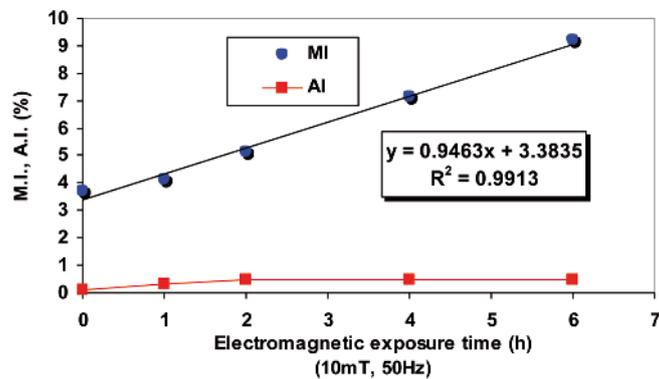


Fig. 5: Mitotic index and chromosomal aberration index after electromagnetic exposure to 50 Hz/100 mT field (y : M.I. (%); x : exposure time (h); R : linear correlation coefficient).

with a high correlation coefficient. A low level of aberrant mitoses was found, between 0.11% and no more than 0.5% so that one could assign these results to spontaneous chromosomal aberrations and not necessarily to the coherent influence of the electromagnetic field action.

4. Conclusion

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.

The exposure of the same biological material to a 50 Hz/100 mT electromagnetic field showed practically no genotoxic effect, as the chromosomal aberration index was only slightly different from that of the control plantlets. On the other hand mitosis stimulation was revealed as a certain consequence of electromagnetic energy absorption.

Linear dose-response functions were proposed for the chromosomal aberration percentage versus radiation exposure intensity as well as for the mitotic index versus electromagnetic exposure time.

Acknowledgements

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References

- [1] SAVAŖKAN C. and TOKER M. C., *Turkish J. Bot.*, 15 (1991) 349.
- [2] EVANS H. J., *Rad. Bot.*, 5 (1965) 171.
- [3] AL-SALHI M., *et al.*, *Nahrung.*, 48(2) (2004) 95.
- [4] OKAMOTO H. and TATARA A., *Environ. Exp. Bot.*, 35 (1995) 379.
- [5] EROGLU Y. *et al.*, *Adv. Biol. Res.*, 1(1-2) (2007) 26.
- [6] XIUZHER L., *J. Nucl. Agricul. Sci. China*, 15 (1994) 53-55.
- [7] STOEVA N., ZLATEV Z. and BINEVA Z., *J. Env. Prot. Eco.*, 2 (2001) 304-308.