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Direct versus indirect radiation action in irradiated vegetal embryos

Research Article

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Abstract: Maize is one of model plants useful for genetic investigations and also very important for its agrotechnical utilizations. Here the genotoxic effects of low dose X-rays and accelerated electrons in maize caryopses was carried out with focus on the influence of water content at the moment of seed irradiation. X-ray photon beam as well as accelerated electrons were provided with 2.40 Gy min^{-1} dose rate. Pre-soaked and dry maize caryopses were irradiated with 0.5-3.0-6.0 Gy. Cytogenetic investigations were carried out based on microscope observations of chromosomes stained by Feulgen method. The mitotic index was found diminished in hydrated samples indicating the negative influence of indirect effects of water radicals. As known the water radiolysis release free radicals that attack biomolecules in addition to the directly absorbed radiation impact. Slight positive influence of 0.5 Gy radiation dose on cell division was evidenced. Chromosomal aberrations were identified like: vagrand chromosomes, C-metaphases, picnotic chromosomes, chromatide bridges. General tendency of aberrant mitoses enhancing was recorded in watered samples - with up to the twice increase for 6.0 Gy radiation dose. The results evidenced the hydration role in monitoring cytogenetic effects of low dose radiations in plant systems -with possible biotechnological applications.

Keywords: *X-rays • Accelerated electrons • Maize caryopses • Hydration level • Mitotic index • Chromosomal aberrations*

1. Introduction

Environmental issues related to human activities focused on pollution in the biosphere have considerably increased over the last few decades. The health risks associated with genotoxic compounds released into the environment from industrial sources, as exemplified by the intensification of background radiation levels generated by the nuclear energy industry, have disturbed the ecosystem and affected living organisms [1]. For instance, in plants, as a result of impact resulting from physical mutagens, reduced levels of germination have appeared, with an associated mitotic index regression and accumulation of chromosomal aberrations that affect the vitality, fertility and productivity of the exposed crops [2]. On the other hand, controlled induced mutations can generate genetic variety and variability, which represents the starting point in selection of desired mutants that have the potential to improve the health, nutrition and wellness of humans. Moreover, much of our understanding of higher organisms at the genetic level is based on studies that utilize induced mutations for gene function analysis [3,4].

When low energy ions are implanted in the absorbent material then the causes of mutation processes are: the direct DNA damaging action due to deposition of ion energy into these molecules and the indirect effects given by energetic ions reactions with water and other cell constituents. The products of such reactions are new molecular fragments (free radicals that tend to

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recombine triggering new abnormal reactions often including DNA injuries) and other new produced molecules that can affect DNA integrity by reacting with it [5].

At the basic level, the effects of ionizing radiation on plants have been analyzed using external radiation sources (X- and gamma-rays, mono-energetic neutrons with various energies, heavy particles, such as protons, nitrogen ions *etc.*) and such investigations have shown that, compared to animals, some plants show higher sensitivity to ionizing radiations [6-8].

The impact of ionizing radiation on living tissues results in the formation of ionized and excited molecules that can cause damage to cellular component and injurious effects of the structure of DNA. Because of the ubiquitous presence of water in biological systems, the products released by the absorption of ionizing radiation in water molecules induce most of molecular damage.

In addition, internal exposure of plants to radiation often occurs through radionuclide accumulation in their tissues, especially in those with actively dividing cells, such as developing and meristematic tissues [9-11].

Ionizing radiations are non-selective mutagenic factors; thus, mutations are randomly distributed in the whole genome of an organism [12]. Since it has been discovered that chromosome damage is the main cause of cell death after irradiation, the detailed assessment of chromosome damage would be useful for the prediction of tumor radiosensitivity [13]. Furthermore, in plants, chromosomal aberrations yielded during cell division, in response to physical or chemical agents, can be used to establish the degree of genome impairment.

The widespread use of radiation in various fields: medicine (for diagnosis, therapy and to sterilization of medical instruments), agriculture and food security, diverse types of industrial processes, continuously increases the risk of the accumulation of radiation in the environment and emphasizes the necessity of assessing associated genotoxicity.

Plants, as basis of food chains of animals and humans, are often used to evaluate the genotoxic and mutagenic potential of risk factors. This is because plants as test systems are able to reveal numerous genetic and chromosomal changes induced by some putative mutagen agents that have not yet been detected using other tests systems either mammalian or non-mammalian but probably acting as mutagens in the human organisms.

Determination of potential genetic hazards, mainly *in situ* in water, soil and air pollutant, would be improved by monitoring mutagens with plants, with an important role in the protection of genetic purity of crop plants and in the melioration of food supply purity [14].

The main purpose of this paper is to evaluate the genotoxic potential of direct and indirect radiation in experimental plant systems – related to the hydration level, as evidenced in root meristems developed from irradiated embryos, by means of chromosomal damage and mitotic irregularities, by analyzing the clastogenic effects (chromosome fragmentation) and aneugenic effects (without DNA damage) induced in maize seedling cells [15].

2. Experimental procedure

2.1 Biological material

Biological material consisted of healthy caryopses of maize (*Zea mays* conv. *dentiformis* Korn., early hybrid *Suceava 108* [16] provided by Agricultural Research and Development Station in Suceava, Romania). This convariety is highly productive and *Suceava 108* hybrid was homologated in 1980 in Romania. Before experiment caryopses were immersed for 3 minutes in freshly prepared NaOCl 3% then washed repeatedly in distilled water to remove disinfectant traces and let to dry for 48 h. Two seed groups were formed: (i) dry seeds for direct irradiation procedure; (ii) soaked seeds that were first wetted for 15 h in distilled water before irradiation.

The irradiated seeds were kept for germination on misted filter paper in the Petri dishes (100 caryopses/dish) in darkens within 20±2°C INCUCELL thermostat. Control samples were maintained, in the same environmental conditions without irradiation.

2.2 Sample irradiation

High energy, *i.e.* low LET X-ray exposure was carried out in a 6 MV photon beam, while accelerated electron exposure was carried out in a 6 MeV electron beam generated by linear particle accelerator VARIAN CLINAC 2100SC type, from the “St. Spiridon” University Hospital, Iasi, Romania.

Dose rate was of 240 cGy min^{-1}, while the exposure times needed to release the established doses in the samples were calculated for *SSD* =100 cm using the formula [17]:

$$t = \frac{D}{\dot{D}\left(z_{\max}, 10, 100, h\nu\right) RDF(A, h\nu) PDD(z_{\max}, A, h\nu) \times 0.005029}$$

where *t* is the irradiation time, *D* is the radiation dose applied to the sample, *Ḋ(zmax)* is the dose rate at the deep *z* in the sample (where the dose is maximum along the central axis of the irradiation field of 10×10 cm^2), *RDF (A, hv)* is relative dose factor, *PDD(1.5, A, hv)* is the percentage depth dose for the irradiation field A.

For the given irradiation geometry (X-ray photons: 6 MV, depth of 1.5 cm, field of 30×30 cm^2, source-to-sample-distance *SSD*=100 cm; electron beam: 6 MeV, depth of 1.5 cm, field of 20×20 cm^2, *SSD* =100 cm) the values of $\dot{D}$, *RDF* and *PDD* resulted from calibration procedures (according to IAEA TRS-398 standard), using type 3D Blue Water Phantom, flat ionizing chamber type PTW Frieburg Markus – for the electron beam, cylindrical ionizing chamber type PTW Farmer – for the photon beam and PTW Unidose electrometer.

In addition, calibration procedures include radiation performance checking. Chosen doses – for application to vegetal samples, were of: 0.5-3.0-6.0 Gy. Corresponding exposure times ranged between 22 s and 184 s.

2.3 Cytological analysis

For cytogenetic analysis, maize roots (not more than 10-15 mm in length), developed from irradiated embryos, were fixed for 24 h in ethyl alcohol: glacial acetic acid (3:1, v/v), at room temperature, and stored in 70% ethyl alcohol. For staining the plant material was hydrolyzed for 10 minutes in 50% HCl and immersed (24 hours, at +4°C) in modified carbol fuchsin [18]. To prepare the microscope slides the terminal root tips (1-2 mm) were removed and squashed in 45% acetic acid [19]. Each sample consisted of six roots meristem each on a microscope slide with ten fields microscopically analyzed using a Nikon Eclipse 600 light microscope. Photos were taken with a Nikon Cool Pix 950 digital camera, 1600×1200 dpi.

Quantitative cytogenetic parameters were established:

MI (mitotic index) = TDC x100/TC; Prophase index = prophase cells x 100/TDC; Metaphase index = metaphase cells x100/TDC; Anaphase index = anaphase cells x 100/TDC; Telophase index = telophase cells x100/TDC; Total aberrations = abnormal cells x 100/TDC; (TC=total analyzed cells; TDC =total dividing cells).

For statistical analysis ten microscope fields on each microscope slide were analysed and average values and standard deviations determined. These data are presented in tables and graphical plots.

3. Results

3.1 Mitotic index and mitosis phases

Zea mays is frequently used as an experimental plant model for *in vivo* estimation of cytotoxic and genotoxic effects of various physical agents, mainly due to its genetic homogeneity, but also due to its practical importance as widely cultivated cereal. In the series of graphs and tables cytogenetic comparisons of qualitative and quantitative parameters that resulted from the analysis of the four data arrays extracted from irradiated maize meristems are presented and further discussed based on literature reports.

The data in Table 1 and Figure 1 present the mitotic activity in root tip cells in relation to the hydration level of irradiated material for both X-ray photons and accelerated electrons. X-rays induced

Dose (Gy)			Total analyzed cells	Prophase index (%)	Metaphase index (%)	Anaphase index (%)	Telophase index (%)
dry caryopses	X-ray	control	5364	61.40	15.35	11.62	11.63
		0.5	5381	64.67	14.27	9.92	11.14
		3.0	4765	61.94	16.26	11.42	10.38
		6.0	5342	66.67	12.69	11.15	9.49
	Electron	0.5	5526	62.37	15.37	11.57	10.69
		3.0	5236	61.60	15.52	11.36	11.52
		6.0	4853	66.32	12.46	10.18	11.04
wet caryopses	X-ray	control	5855	69.32	11.73	10.23	8.72
		0.5	4860	63.77	14.10	12.62	9.51
		3.0	6724	68.31	12.43	9.84	9.42
		6.0	5354	64.64	14.14	10.75	10.47
	Electron	0.5	6118	62.85	15.12	11.58	10.45
		3.0	6720	65.52	13.03	11.45	10.00
		6.0	5233	66.90	14.59	10.68	7.83

Table 1. Cytogenetic parameters in maize root tip meristems, after caryopses (dry and pre-soaked) exposure to the same doses of X- ray photons and accelerated electrons.

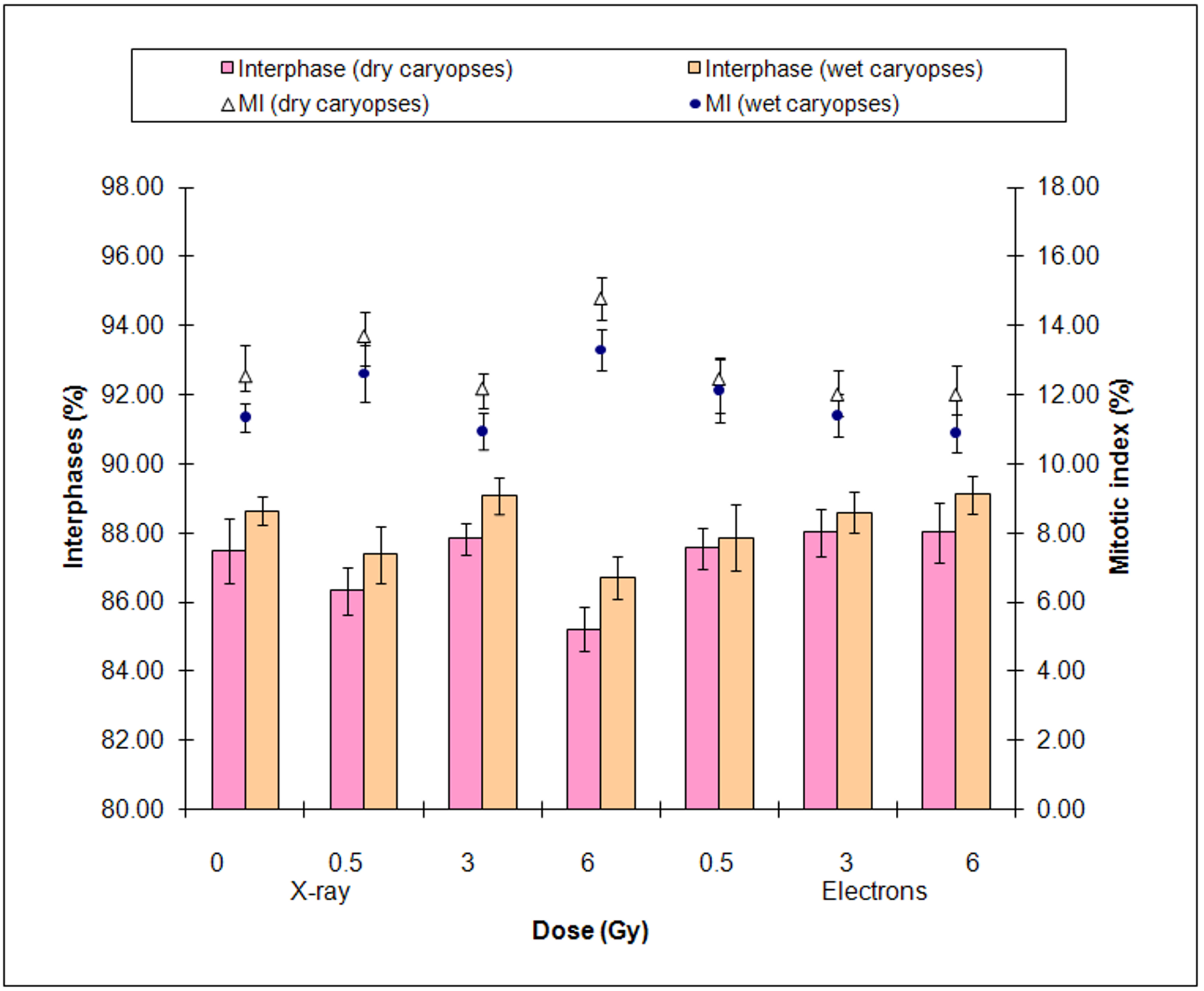

Figure 1. Mitotic index and interphase percentage in irradiated maize root meristems.

significant changes in the mitotic index for both types of maize samples: dry and respectively pre-soaked caryopses. Cell division rates, expressed by mitotic indexes, appear to be always lower in the wet seed experimental variants compared to dry seed variants – for each radiation dose. Non-linear dose-response relationship was shaped for X-ray irradiation; in the case of accelerated electrons the mitotic index is also smaller in soaked seeds for all radiation doses but the graph trend is different, revealing linear decrease to the dose enhancing. The proportion of interphase cells exhibits opposing variation in all cases – as expected. The greatest quantitatively detailed information on mitotic responses to radiation is shown in Table 1 and presents additional cytogenetic parameters (indexes) *i.e*. cell frequency in different mitotic phases. As could be seen also in Figure 2 the prophase index is the highest in all experimental variants – over 60% either in control samples or in the irradiated samples.

Also the metaphase index is relatively high, up to 16%, while anaphase and telophase cell indexes do not exceed approximately 12% and 11%, respectively. The most evident differences between dry and wet seeds response to irradiation consist in the lower frequencies of anaphases and telophases, anaphase indexes remaining higher than telophase ones.

3.2 Aberrant mitosis frequency; ana-telophases (A-T) and metaphases (M)

In Figure 3 the results for the frequency of aberrant mitosis are presented. In dry caryopses X-ray irradiation up to the dose of 3.0 Gy led to progressive accumulation of cells with various types of chromosomal aberration, while for the highest applied dose a diminution was recorded. Similarly in dry seeds exposed to the electron beam the highest accumulation of aberrant mitoses occurred at 0.5 Gy. The net enhancement of aberrant mitoses in wet samples irradiated with 6.0 Gy was

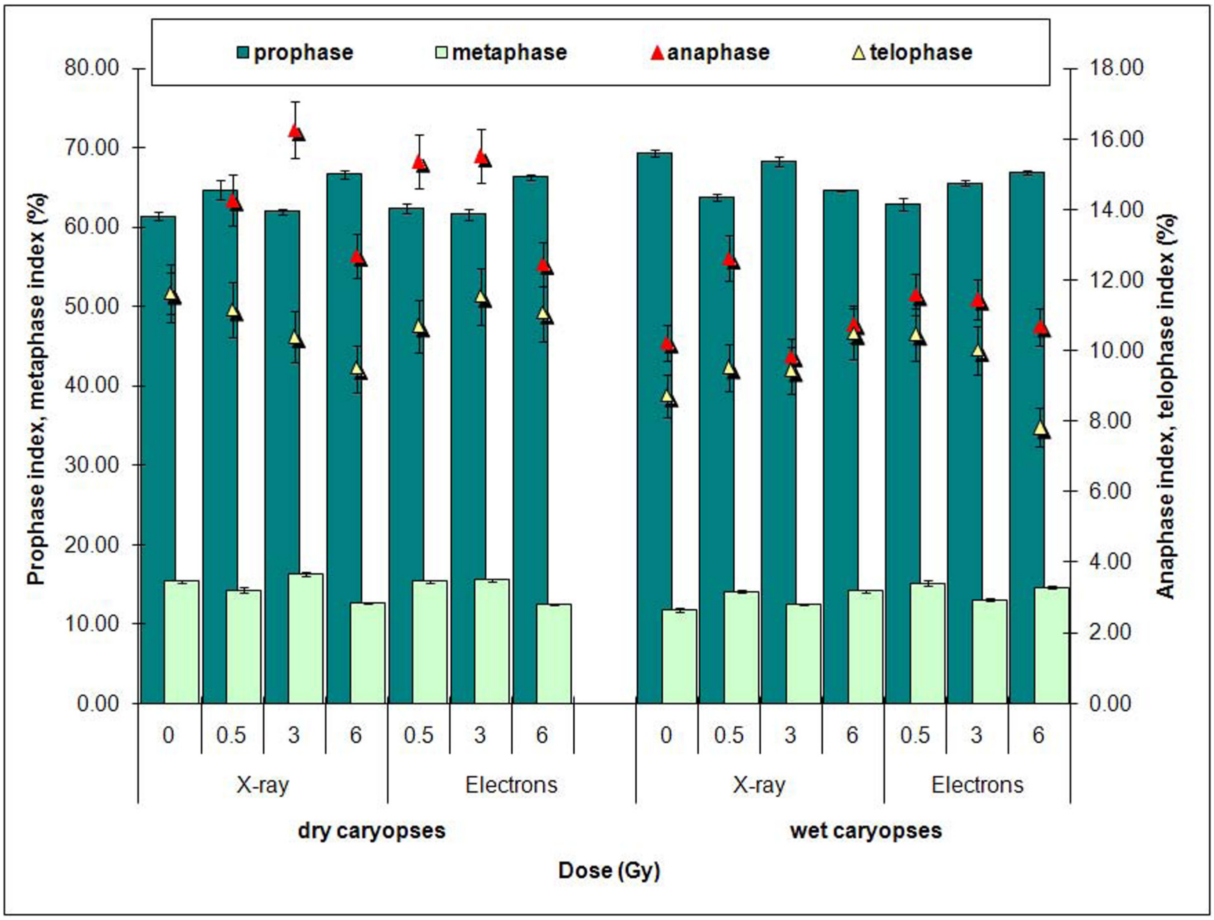

Figure 2. The effect of irradiation on the percentage of the cell mitosis phases.

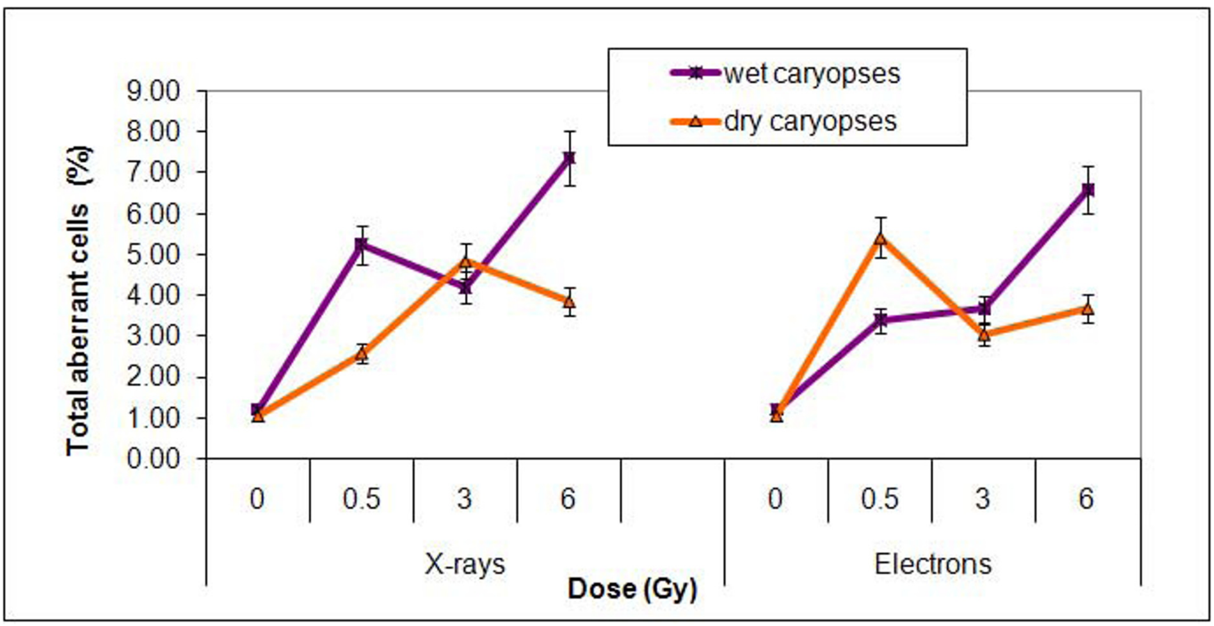

Figure 3. Percentage of aberrations in irradiated maize samples.

noticed in both X-ray and electron beam irradiation (Figure 3); this effect could be due also to mitotic index relative diminution – so that higher numbers of dividing cells enhanced the calculated percentage of aberrant mitoses.

In ana-telophase the most frequent chromosomal aberrations were the simple and multiple bridges (Figure 4a, b respectively), followed by laggard and/or vagrant chromosomes (Figure 4c) (chromosomes delayed in separation of anaphase cells) and acentric fragments (small chromosome parts without centromeres). A reduced number of complex aberrations was also noticed in certain ana-telophase cells including two or more associated simple aberrations; so associations of simple and multiple bridges were observed as well as chromosomal fragments and lagged/vagrand chromosomes.

In dry seeds exposed to X-ray irradiation the percentage of A-T bridges (Figure 5) is even lower than that obtained as a result of spontaneous impact of environment gradients (in the control, non-irradiated samples), but in wet seeds the increase of simple and multiple bridge number is evident (up to 2%) for X-ray doses of 3.0 and 6.0 Gy. In the case of electrons the total bridge level is around 1% (except for wet seeds at 0.5 Gy where 1.6% was reached) and the influence of water seems to lead also to opposing slight changes in dry seeds compared to wet ones.

In Figure 6 the variation of ana-telophase aberrations with the dose and hydration level could be seen. It is evident that aberrant metaphases exhibit higher percentages than aberrant ana-telophases both for X-ray treated samples (Figure 6a) and for electron irradiated samples (Figure 6b) either for dry and wet conditions. In the wet seed samples exposed either to X-rays or to electrons, the dose increase resulted in higher percentages of aberrations than in non-irradiated seeds, with higher level of aberrant metaphases compared to aberrant ana-telophases. In the case of dry seeds the dose of 3 Gy led to lower level of aberrant

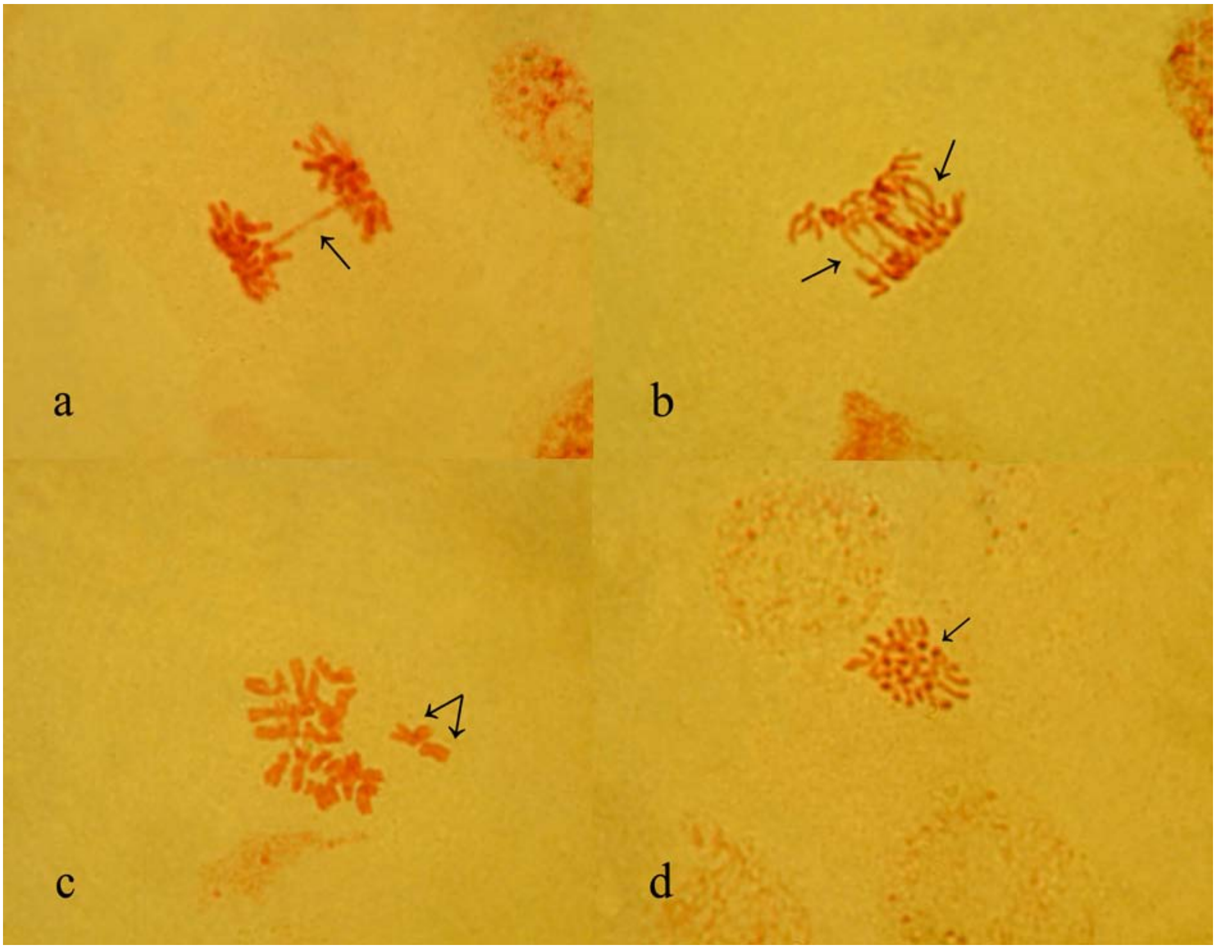

Figure 4. Chromosomal aberrations identified in the analyzed samples; a) simple bridge; b) multiple bridge; c) vagrand chromosome; d) picnotic chromosomes.

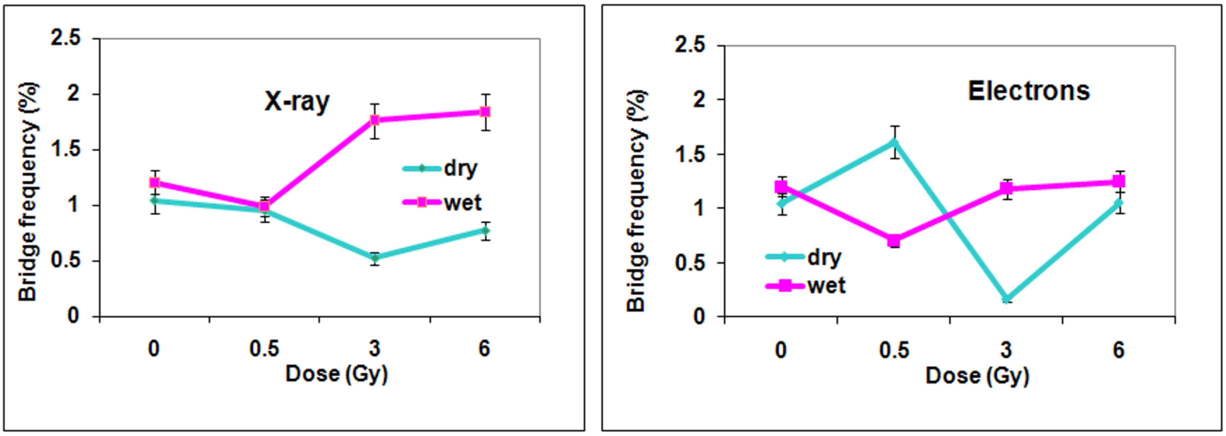

Figure 5. Total percentage of bridges in the analyzed root meristem cells.

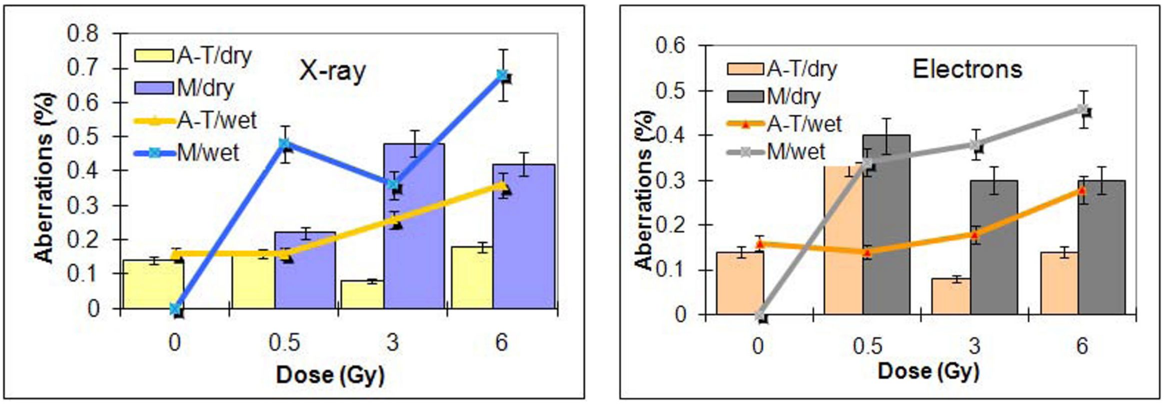

Figure 6. Aberrant cell percentage in ana-telophases (A-T) and metaphases (M).

ana-telophases than in control samples for both X-rays and electron beams.

Ana-telophases with aberrations were recorded not only in the irradiated seeds but also in control ones; around 0.015% of the dividing cells in the control samples exhibit aberrations in ana-telophase which are probably caused by environmental gradients of physical or chemical nature that could not been perfectly controlled during seed storage. This is not surprising as the frequency of spontaneous mutation varies from gene to gene and from organism to organism, ranging for an individual gene from one mutation per 10^5 genes to one mutation per 10^7 genes in a generation [4].

Aberrant cells in ana-telophase present rare lagging as well as vagrant chromosomes – the observed level are less than 1% (Table 2) – with the highest frequency corresponding to 6.0 Gy in wet seed samples. Some metaphase aberrations consistent with vagrant chromosomes (Table 2) are known to result from perturbations at the kinetochore level with consecutive displacement of chromosomes outside of the equatorial plate - closer to or further from cell poles. In a small number of metaphase cells, the chromosomes appeared to be dispersed in the cytoplasmic cell mass, similar to that seen with colchicine treatment – that is the C-metaphase aberration (Table 2) associated with radiation mimics the effect of colchicines, characterized by over condensation or spiralisation of chromosomes and the spindle damage. C-metaphases exhibit the highest accumulation – approximately 3% for wet seeds treated with X-rays.

Also several treatment situations generated picnotic chromosomes (Figure 4d); these were noticed during cytogenetic analysis of aberrant metaphases (Table 2) formed from condensed and strongly spiralized sister chromatid pairs of chromosomes with consecutive drastic reduction of their size. Picnotic chromosomes are less frequent with approximately 1.5% in dry seeds exposed to a 3.0 Gy dose of X-rays.

Metaphase vagrant chromosomes are more frequent in wet samples than in dry ones both for X-rays and electrons with a maximum of approximately 2.5%

	Aberrations in Ana-telophase						
Dry seeds		X-ray			Electrons		
	Control	0.5 Gy	3.0 Gy	6.0 Gy	0.5 Gy	3.0 Gy	6.0 Gy
Simple bridges (%)	0.29	0.27	0	0.13	0.58	0	0.52
Multiple bridges (%)	0.74	0.68	0.52	0.64	1.02	0.16	0.52
Lagging chromosomes (%)	0	0.14	0.17	0.38	0.44	0.48	0
Vagrant chromosomes (%)	0	0	0	0	0.29	0	0
Wet seeds	Control	0.5 Gy	3.0 Gy	6.0 Gy	0.5 Gy	3.0 Gy	6.0 Gy
Simple bridges (%)	0.30	0.66	0.68	0.99	0.14	0.52	0.71
Multiple bridges (%)	0.90	0.33	1.08	0.85	0.56	0.66	0.53
Lagging chromosomes (%)	0	0.16	0	0.71	0	0	0.71
Vagrant chromosomes (%)	0	0	0	0	0	0	0.53
	Aberrations in metaphase						
Dry seeds		X-ray			Electrons		
	Control	0.5 Gy	3.0 Gy	6.0 Gy	0.5 Gy	3.0 Gy	6.0 Gy
Vagrant chromosomes (%)	0	0.14	0	0.38	0.98	0.95	2.69
C-metaphase (%)	0	1.09	2.60	2.31	3.11	1.49	2.12
Picnotic chromosomes (%)	0	0.27	1.56	0	0	0	0
Wet seeds	Control	0.5 Gy	3.0 Gy	6.0 Gy	0.5 Gy	3.0 Gy	6.0 Gy
Vagrant chromosomes (%)	0	1.02	0.64	0.70	0.71	0.79	1.42
C-metaphase (%)	0	2.04	1.76	1.93	1.84	1.70	2.14
Picnotic chromosomes (%)	0	0	0	0	0	0	0.53

Table 2. Chromosomal aberrations in ana-telophase and metaphase for dry and wet seeds.

in wet samples exposed to X-rays. This result is in accord with the previous observations [20] regarding actively dividing cells, wet seeds being found to be more sensitive to radiation damage than quiescent tissue or dry material, any diminution of the ability for genetic repair increases the chances of chromosomal aberration and gene mutation.

4. Discussion

This study compares the radiation direct and indirect actions in irradiated vegetal tissues of maize by estimation of an array of cytogenetic parameters. Due to the influence of water free radicals generated by tissue irradiation *i.e.* radiation indirect action, the experiment was carried out on seeds with different water content: dry seeds -where indirect radiation action mediated by water free radicals is supposed to be lower and soaked ones where the role of water free radicals is expected to be higher. This could be estimated by comparing the total radiation effects at genetic level in dry versus wet seeds. Thus – considering also the low LET radiation used in this study [21,22] - it is postulated that the direct radiation action dominates in dry biological material with lower water content, while indirect action could be observed mainly in hydrated tissues. The quantitative cytogenetic parameters measured were basically the mitotic index and total aberration frequency but also the frequency of cells in different phases of mitosis (prophase index, metaphase index *etc.*, Table 1), as well as the percentages of chromosomal aberrations in different mitotic stages.

As shown in Figure 1 for X-ray irradiation, cell division stimulation in both wet and dry seeds was evidenced based on the increased mitotic indexes for lowest radiation dose compared to control non-irradiated seeds while inhibition was recorded for highest dose tested in this experiment– in both dry and wet caryopses. It is remarkable that the mitotic index was generally lower in wet seeds than in dry ones.

Mitosis stimulation observed for X-ray low dose – followed by mitosis rate decreasing for higher doses - was in accord with other reports that have already convincingly emphasized this behavior in plants, mammals, bacteria and fungi [23]. For electron irradiation of maize caryopses this was not evidenced as the mitotic index decreased for both seed series. Complementary interphase cell percentage appeared to be slightly increased in the irradiated samples compared to the control ones.

In contrast it is the mitostatic effect of maize irradiation that was emphasized for all the electron irradiated samples as well as for higher doses of X-rays, of 6.0 Gy (Figure 1). The higher mitostatic effect was recorded in pre-soaked seeds – as seen for the dose of 6.0 Gy especially (where the difference between the mitotic index values is highest – of about 2%), and this finding is concordant with other reports [24] where it was demonstrated the higher radiation sensitivity of hydrated water seeds. It seems that additional indirect radiation actions – through the intermediate free radicals potential released following water radiolysis, could be seen from the changes in the mitotic index that was diminished in all soaked samples compared to dry ones. The hydration does not change the shape of the graph; the dose-response graphs of mitotic index have similar trends for dry and wet seeds: linear decreasing for accelerated electrons but non-linear trend for X-rays. Also a non-linear dose effect relationship was found by other authors; for example in [25] the authors evidenced non-linear dose-responses in rice irradiated with low doses of carbon ions (0.02-0.2-2.0 Gy) due to the mitogenetic phenomenon for the lowest doses followed by mitostatic effect at higher radiation dose (20 Gy), while in [26] similar trends were revealed in barley exposed to gamma rays (0.01 – 1.0 Gy). Accumulation of metaphases and anaphases following absorption of radiation energy *i.e.* the increased anaphase and metaphase indexes compared to control non-irradiated seeds (Figure 2) could be assigned to the impact of radiation on centromeric histone proteins, which ensure the suitable function of kinetochores [27] that cause chromosome blockage in the metaphasic plate and further the delay of longitudinal cleavage of the two chromatids and obstruction of migration to the cell poles.

In Figure 2 the influence of water is evident from the lower value of anaphase and telophase indexes for the wet seeds compared to dry ones – about 2.5 % diminution. Thus the free water radicals that were considerably more numerous in the wet tissue have favored radiation effects on the mitotic spindle (blocking chromosomes in metaphase plate with metaphase cell percentage increasing) so that fewer cells pass in the next phases of mitosis – anaphase and telophase; moreover this could result in an aneugenic effect (aneuploidy-inducing) [15].

According to Figure 3 the water influence on aberration frequency is mostly remarkable for the highest radiation dose, of 6.0 Gy tested in this experiment – both for X-rays and for accelerated electrons, since the total percentage of chromosomal aberrations was enhanced almost twice in wet seeds (6.5% for electrons and 7.3% for X-ray) compared to dry ones (3.7% and 3.9%, respectively). Evidently the indirect radiation effects have dominated the biophysical and biochemical molecular changes that finally led to DNA fragmentation or its synthesis perturbation with cellular consequences consisting in chromosomal aberrations. It is possible that the direct action of radiations could break down hydrogen bonds involved in secondary and tertiary structures of nucleic acids or affect enzymes associated with genomic integrity with concomitant damaging effects on the chromosomes; this occurs also in the case of hydrated seeds like for dry ones. Indirect radiation actions mediated by dissociated water molecules are also present in both cases, since dry seeds are not entirely lacking water, but simply have a markedly lower hydration level. On the basis of this assumption the extent of cytogenetic effects generated by indirect radiation on wet tissue will be higher than in dry cells. Water radiolysis always generates free radicals of hydroxyl and hydrogen that further reacts with biomolecules involving also oxygen, mainly ROS – reactive oxygen species. This supposedly occurs for X-rays as well as for accelerated electrons with possible peculiarities related to the LET differences and/or to the nature of radiation – electromagnetic or corpuscular. For X-ray exposure the radiative absorption of photon energy results in molecular ionization and dissociation phenomena while for electron beam the electric Coulombian forces led to the same qualitative changes; it is possible that quantitative differences observed in some samples between X-ray and accelerated electron effects are related to the nature of radiation more than to the water influence.

To give an interpretation related to bridge like aberrations (Figure 5) previous studies [28] need to be mentioned as they focused on *in situ* hybridization and demonstrated that chromosomes involved in bridges are implicated in a significantly higher level of structural damage compared to other chromosome aberrations. The results presented above are in agreement with conclusions [29] that suggest changes in ana-telophase and metaphase are both major signs of mutational processes. The mutagenic potential of radiation is known from spontaneous mutation observation - although other environmental gradients may also contribute; according to some estimations spontaneous mutations frequency in superior plants is of 2×10^{-7} mutations/locus/gamete

[30]; for example in maize this frequency ranges between 0.1×10^{-5} and 49.2×10^{-5} mutations/gamete for eight maize loci [31].

Bearing in mind the importance of yielding superior features in agrotechnical plants the use of radiations has been considered as a biotechnological tool for inducing large scale individual variability through controlled radiation induced mutations to generate a wide scope of successful selection. The investigations presented herein revealed the positive role of hydration levels in the increase of chromosomal aberrations in a recognized plant experimental system.

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