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IRON OXIDE NANOPARTICLES COATED WITH ASPARTIC ACID AND THEIR GENOTOXIC IMPACT ON ROOT TIP CELLS OF *ZEA MAYS* EMBRYOS

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Abstract. The goal of the present study was to evaluate the genotoxic influence of iron oxide nanoparticles coated with aspartic acid (AIONPs) with 9.3 nm physical diameters on the root tip cells of *Zea mays* seeds. The seeds germination occurred under AIONPs presence by addition into the germination substrate of AIONPS aqueous solutions with different volume fractions (range between 20 and 400 μ/l). For all samples (control and treated samples) indexes of cytogenetic assays were established. The mitotic division of cells seems was stimulated under AIONPs influence (up to 68%) with a low rate of aberrant cells occurrence (up to 1.42%).

Key words: iron oxide, magnetic nanoparticles, mitotic division, genetic impact, chromosomal aberrations, cytogenetic assay.

1. INTRODUCTION

Environmental contamination with unsafe chemicals is increasing which affect constantly the balance of ecosystems. The heavy metals and metal oxide accumulations from the water sources and agricultural soils lead to bioaccumulation of metals in different crops in different part of plants. Nowadays, the fast development of nanotechnology, using mostly nanoparticles with sizes more less than 100 nm, bring to increased concern in relation to the nanoparticles impact on the environment as well as on plant and animal organisms [1]. Also, the plants, important component of the ecosystem, can provide a possible way for nanoparticles transport from the environment, conducting to their accumulation into the human alimentation chain. The researches on nanoparticles field has become more attractive due to its unique properties contrary to other materials, that leading to their application in a large number of research domains, both in technology and in biology and medicine. In the last period due to the more and more utilization of the magnetic nanoparticles in treatments or medical diagnostics, the interest for the biological applications of iron oxide nanoparticles has increased [2]. The magnetic nanoparticles can be manipulated by an external magnetic field gradient due to

their magnetic properties. Iron oxide nanoparticles with magnetic properties are used for a large range of biomedical applications like drug delivery, thermal ablation therapy, magnetic resonance imaging, hyperthermia of cancer cells, immunoassay, magnetic separation of cells, and *in vivo* cells tracking [3–6]. Iron oxide nanoparticles offer interesting possibilities for researches in the biotechnology field, due to the small sizes between few nanometers and tens of nanometers. There are an increasing number of studies focused to studying the interaction of iron oxide nanoparticles with biological systems, but the published experimental data aren't consequent; these variations could be due to the differences in nanoparticles properties (such as size, shape, surface coating, surface charges, physical properties, so on) or different experimental schedules [7]. Even if the amount of research on the toxicity of nanoparticles to animals and bacteria had increased, a limited number of studies evaluate the effects and potential toxicity of nanoparticles at plants [8–13]. Many research studies are dedicated to the influence of magnetic nanoparticles on the germination process, with both inhibitory and stimulatory effects [14–17]. Genotoxicity is one of the most disruptive effects that nanoparticles could induce on plants. Although the number of researches in plant genotoxicity field is lower, the all reported results revealed genotoxic effects of various types of nanoparticles in various plants species, with different levels of influence [18–22]. The underlying mechanisms responsible for the toxic effects of the nanoparticles are not yet understood.

The aim of this study was to evaluate the genotoxicity of the iron oxide nanoparticles coated with aspartic acid (AIONPs) to meristematic tissues level of *Zea mays* root tips by cytogenetic tests. Cytogenetic tests are suitable for identifying the disruptive effects of nanoparticles presence in different concentrations. Present experiment was carried out during March and April 2019 in the university laboratories in Sibiu, Romania.

2. MATERIALS AND METHODS

2.1. IRON OXIDE NANOPARTICLES

The aqueous suspensions of AIONPs were synthesized by the co-precipitation of iron ferric and ferrous chloride in alkali medium at about 80°C and than stabilized with L-aspartic acid ($C_4H_7NO_4$), following the protocol described in [23]. Technological process of the AIONPs preparation in Fig. 1 is presented.

The mean diameter of the AIONPs was estimated from TEM (transmission electronic microscopy) imaging analyses of 10^4 diluted aqueous suspensions (a TESLA device having 1.0 nm resolution was used). TEM images were analyzed using ImageJ Software for nanoparticles sizes attainment. Six different TEM images were used, analyzing 700 nanoparticles in total. The obtained data was used to plot the histogram and obtain the mean size of nanoparticles and standard deviation. According with TEM images, the AIONPs sample has mean diameter of

about 9.3 ± 2.5 nm, with spherical shape and dimensional distribution ranging between 3.2 and 16.7 nm. In Fig. 2 a TEM image and size distribution of AIONPs are presented.

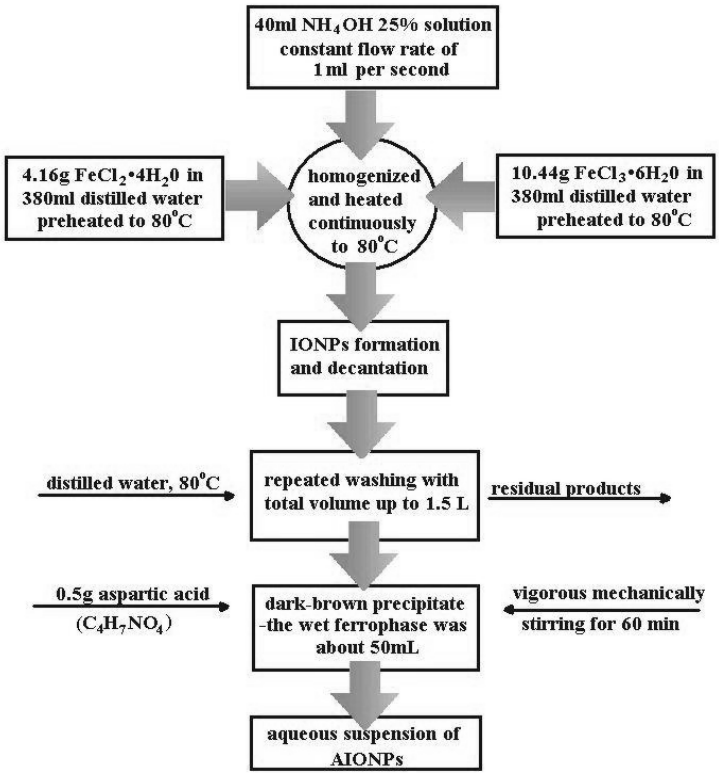

Fig. 1 – Schematic presentation of AIONPs synthesis technology.

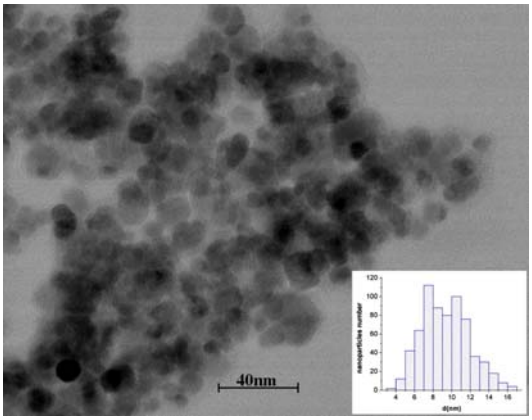

Fig. 2 – TEM image and size distribution of AIONPs (scale bar = 40 nm).

AIONPs native suspension had the saturation magnetization of 50.2emu/g (the experimental data were recorded by vibrating sample magnetometry technique using a MicroMag 2900 model device at room temperature).

AIONPs sample was choose for this experimental study due to its particular biological interest; in plants the aspartate is the precursor to several amino acids, while the iron oxide nanoparticles (magnetite or maghemite) are biocompatible compounds [5].

2.2. VEGETAL MATERIAL

The maize seeds (*Zea mays*) were chosen as biological material related to its economic importance for agriculture and food industry. *Zea mays* plant is one of the most preferred species for genetic investigation due to the higher sensitivity to the mutagenic factors exposure [24]. Experimental lot of *Zea mays* seeds with uniform genetic features were insured from local source. Twenty seeds for each sample were let to germinate in Petri dishes on moistened filter paper with 15ml aqueous solutions of AIONPs with different volume fractions, in a controlled laboratory room at 24°C, in darkness. The AIONPs aqueous suspensions with volume fractions of 20 – 40 – 80 – 160 – 320 – 400µl/l were used. The control sample was let to germinate in the same environmental conditions but substrate was only supplied with distilled water (15ml). Two-three days old germinated seeds were selected based on root length (1–2 cm) for cytogenetic analysis.

2.3. SLIDE PREPARATION AND CYTOGENETIC ANALYSIS

Roots from control and AIONPs treated samples of *Zea mays* germinated seeds were fixed in Carnoy solution (1 glacial acetic acid / 3 absolute ethanol); v/v for 24 hours and stored in 70% ethanol at 4°C in refrigerator. Squash method combined with Feulgen technique was used for root samples preparations [25]. The root tips hydrolyze was made in 1N HCl and 50% HCl. Then these were transferred in Carr dye (modified carbol fuchsin) for at least 24 hours at 4°C to obtain selective coloration of chromosomes (purple). All used reagents were reactive grade (J.T. Baker, Holland; Sigma-Aldrich, UK and Merck, Germany). For a microscopic slide obtain, the terminal root tip (1 mm) was cut off and crushed onto slide in a drop of 45% acetic acid. Five temporary slides were prepared for each experimental sample to asses the percentage of mitotic index (M.I.) and chromosomal aberrations index (A.I.). M.I. is able to give the percentage of dividing cells in every sample while A.I. represents the percentage of aberrant cell divisions. Each slide was examined for counting the cells in different division phases (either normal or abnormal ones). At least 2,000 cells of each root meristeme tissue and over twenty microscopically fields per slide was analyzed, by the same operator using an optical trinocular microscope (Euromex IS 1153-EPL

model, Holland). A digital photo camera (FUJI–FinePix S5100 model) was used for take of the abnormal cells microphotographs. The quantitative parameters, M.I. and A.I. were calculated as: M.I. (%) = (TD × 100)/TC; A.I. (%) = (TA × 100)/TC (TC = total number of analyzed cells; TA = total number of abnormal cells, TD = total number of divided cells).

2.4. STATISTICAL ANALYSIS

Experimental data were worked using Microsoft Excel soft package and Statistica v.7.0, to evaluate reliability of AIONPs induced changes. Results from microscopic analyses of five slides for each experimental case (control and IONPs treated samples), are shown as mean value ± standard deviation. Descriptive statistic parameters were estimated for both M.I. and A.I. for each experimental sample. The statistical significance between control and AIONPs treated samples were analyzed using one-way analysis of variance (ANOVA), the significance being defined by a probability level of $p < 0.05$. Graphic dependences were displayed in ORIGIN 6.0.

3. RESULTS AND DISCUSSIONS

Genotoxicity could be defined like some disorders or damages of the somatic cells or DNA or alterations of the mitotic division phases. The risk of the iron oxide nanoparticles influence on disorder of the genetic material was estimated by means of chromosomal aberration index in the meristematic cells of *Zea mays* embryos.

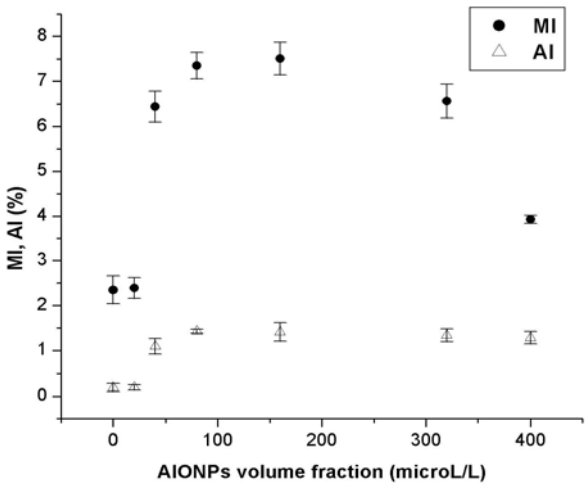

Fig. 3 – Mitotic index (M.I.) and aberrations index (A.I.) for all experimental samples in relation with AIONPs volume fraction added in germination substrate (μl/l).

In this study, the results indicated that AIONPs presence during the germination process of seeds was able to modify the mitotic division in root tip cells. AIONPs with 9.3 nm size were able to stimulate the mitotic division activity, mitotic index being increased up to 68% than control one. AIONPs presence caused an increase (up to 86%) in the frequency of abnormal cells, also. In Fig. 3 is presented the variations of the mitotic index and the aberrations percentage index with AIONPs volume fraction added in the seeds germination substrate. Each value (mean with error bar) of indexes represents the mean for five replicates (slides) in each experimental sample with standard deviation. All cytogenetic analysis results (M.I. and A.I. values) have been statistically significant in comparison with control, for all exposed samples ($p < 0.05$).

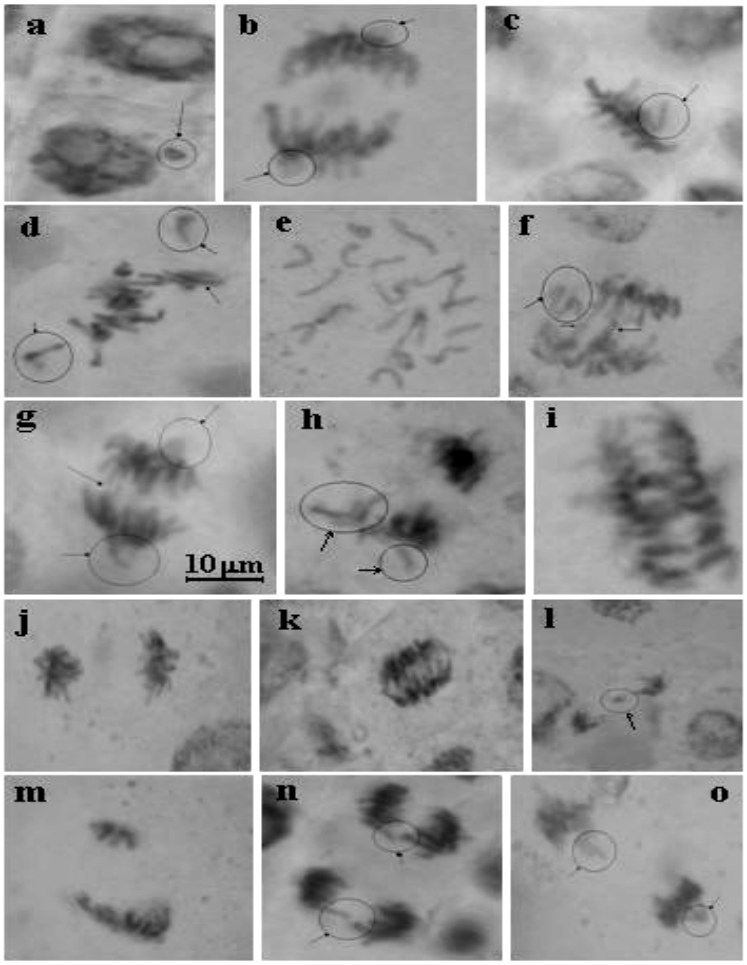

Fig. 4 – Some example of abnormal cells found in root tips cells of AIONPS treated seeds of *Zea mays*.

The more observables chromosomial aberrations detected in root tip tissues of AIONPs treated samples were interchromatin bridges, vagrants, laggard chromosomes, delayed anaphases, ring chromosomes, anaphases with star effect, fragments of chromosomes and micronuclei. Micro-photographs of some abnormal cells observed during the microscopic analyses are presented in Fig. 4.

In Table 1 are presented the abnormal cells types observed in the roots meristeme treated with AIONPs. The abbreviations used in Table 1 are: S – experimental sample; VF – the AIONPs volume fraction; TC = total number of analyzed cells for each sample; TA = total number of abnormal cells; TD = total number of divided cells; Mn – micronuclei; Vg – vagrant chromosomes, Lg – lagging chromosomes, DA – delayed anaphase, Cm – C mitosis, Bg – chromatin bridges, Bk – break of chromosomes, fragments, Rc – ring chromosomes, Oth – others aberration types.

Table 1

Cytogenetic analyses results with abnormal cells types

VF (µl/l)	**TC**	**TD**	**TA**	**Chromosomal abnormal cells**								
				Mn	**Vg**	**Lg**	**DA**	**Cm**	**Bg**	**Bc**	**Rc**	**Oth**
0	10115	238	20	2	6	2	0	0	5	0	1	4
20	10900	258	22	4	2	6	3	0	3	2	0	2
40	25576	1645	281	1	50	61	24	0	43	31	16	55
80	15956	1172	227	1	54	47	12	0	24	24	12	53
160	16380	1225	235	2	43	49	8	0	29	30	12	62
320	14525	956	189	4	53	34	5	0	30	17	9	37
400	12385	488	152	0	23	59	7	10	17	6	0	30

Micro-photographs of some abnormal cells observed during the microscopic analyses are presented in Fig. 4 as follows: (a) – micronuclei in interphase; (b) – anaphase with ring chromosome and vagrant chromosome; (c–d) – laggard chromosomes in metaphase; (e) – C mitosis; (f) – anaphase with interchromatin bridges and vagrant chromosomes; (g) – anaphase with ring chromosomes and fragments; (h) – anaphase with vagrant and fragments; (i) – delayed anaphase; (j) – anaphase with star effect; (k) – anaphase with multiple interchromatin bridges; (l) – apolar anaphase with vagrants and fragments; (m) – asymmetric anaphase; (n) – broken interchromatin bridges in anaphases; (o) – anaphase with vagrant and ring chromosome; the arrows indicate particularly, the specific chromosomal aberrations.

For all values of volume fraction of the AIONPs added in the germination substrate were obtained enhanced values (statistically significant) than control ones, of the both cytogenetically indexes of interest in this experimental study. It was observed that the AIONPs suspensions at low volume fractions (40 – 80 – 160 – 320 µl/l) tend to have beneficial impact on vegetal embryos and improve the mitotic division process with a low percentage of abnormal cells induced. By correlation analysis, Kendall's tau correlation coefficient of 0.809 ($p < 0.05$) and respectively, Pearson

coefficient of 0.877 ($p < 0.01$) were obtained between the results data of the cytogenetic indexes (M.I. and A.I.), revealing a positive correlation between of the values of indexes. For the largest volume fractions used in this experiment, there was a tendency to decrease the mitotic index than the values obtained for lower volume fractions, but these values are higher than those obtained for the control sample. In case of the smallest value of the AIONPs volume fraction the variations of MI and AI were low. It seems that value of 20 μl/l is a very small value for volume fraction of the AIONPs added in germination substrate of the *Zea mays* seeds and its influences are not very important as against those were obtained for the others volume fraction values used in this experiment. A significant increase of the abnormal cells percentage (A.I.) was recorded for AIONPs treated samples in comparison with control group (up to 86%). The laggard chromosomes in metaphase or anaphase are one of the major abnormal cell type noticed in the present experimental study. Also, vagrant chromosomes are another frequently aberration observed. These types of abnormal cells are physiological aberrations. It seems that AIONPs presence in substrate of seeds germination could influence the division of centromere.

Similar results have also been obtained for citric acid stabilized iron oxide nanoparticles, synthesized in our laboratories. The mitotic division was stimulated, with significant increases in the mitotic index especially for low volume fractions of solutions of iron oxide nanoparticles coated with citric acid [25]. Also, the percentage of chromosomal aberrations induced by the presence of Fe_3O_4 nanoparticles in the germination substrate of *Zea mays* seeds was small (up to 0.53%) [26]. But there have also been reported opposite results for other types of magnetic nanoparticles. In case of treatment with relatively low volume fractions of the $ZnFe_2O_4$ and $CoFe_2O_4$ nanoparticles, applied to sunflower seeds, considerable diminished mitosis rate was evidenced while the percentage of abnormal cells was remarkably enhanced [21]. In other research study it was used cobalt oxide nanoparticles for treatment of the *Sesbania cannabina* seeds and it was obtained a slight decrease in the mitotic index and 10% increase in the aberrations index [27]. Considering the results of other scientific studies, it seems that the influence of water-based magnetic nanoparticles on the plant embryos tends to depend on the type of nanoparticles used, the type of coating agent used in the nanoparticle synthesis and the treated plant species.

Iron is required to maintain viability and to assist the growth of almost all kinds of cells, its presence being mandatory for cellular proliferation [28]; depletion of iron generally stops the cell cycle. In majority of the research study it is postulated that iron oxide nanoparticles are able to cross the plasma membrane by endocytotic [29] or non-endocytotic [30] pathways, reaching a direct access to the cytoplasm and other cellular compartments. Due to the fact that iron deficiency is a major health problem, more and more is suggested that the iron oxide nanoparticles to be used like iron supplementation due to their high bioavailability, good stability, low side effects and lack of taste and color variations of the foods [31].

Even though iron is an essential nutrient, it can cause cells alteration when present in excess [32]. Thus, experimental studies are required to establish the level of iron oxide nanoparticles necessary to obtain optimal influences by increasing of the cell proliferation level with a low percentage of abnormal cells.

4. CONCLUSIONS

The magnetic nanoparticles genotoxic effects evaluation is one of the modern research topics and of high interest which could contribute to the knowledge and understanding of the influences of nanoparticles released into the environment. The present study provide supplementary and useful research results about the genotoxic effect of iron oxide nanoparticles by evaluating mitotic index and chromosomal abnormalities in the meristematic root cells of *Zea mays*, being of interest for environmental issues associated with estimation of magnetic contamination risk. Presence in the *Zea mays* seeds germination substrate of the iron oxide nanoparticles coated with aspartic acid with 9.3 nm sizes can induce a low percentage of chromosomal aberrant cells in root tips (up to 1.42%) and increase of the mitotic index (up to 68% than control one). These results might be helpful in expanding the research on mechanisms by which plant tissue respond to iron oxide nanoparticles presence and to optimize the presence of nanoparticles in the germination substrate of the seeds to achieve positive influence results on the proliferation of cells.

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