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Research Article

Plant Explants Grown on Medium Supplemented with Fe_3O_4 Nanoparticles Have a Significant Increase in Embryogenesis

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Development of nanotechnology leads to the increasing release of nanoparticles in the environment that results in accumulation of different NPs in living organisms including plants. This can lead to serious changes in plant cultures which leads to genotoxicity. The aims of the present study were to detect if iron oxide NPs pass through the flax cell wall, to compare callus morphology, and to estimate the genotoxicity in *Linum usitatissimum* L. callus cultures induced by different concentrations of Fe_3O_4 nanoparticles. Two parallel experiments were performed: experiment A, where flax explants were grown on medium supplemented with 0.5 mg/l, 1 mg/l, and 1.5 mg/l Fe_3O_4 NPs for callus culture obtaining, and experiment B, where calluses obtained from basal MS medium were transported into medium supplemented with concentrations of NPs identical to experiment A. Obtained results demonstrate similarly in both experiments that 25 nm Fe_3O_4 NPs pass into callus cells and induce low toxicity level in the callus cultures. Nevertheless, calluses from experiment A showed 100% embryogenesis in comparison with experiment B where 100% rhizogenesis was noticed. It could be associated with different stress levels and adaptation time for explants and calluses that were transported into medium with Fe_3O_4 NPs supplementation.

1. Introduction

Development of nanotechnology and application of different nanoparticles (NPs) in industry have essentially increased [1, 2] and worldwide investment in nanotechnologies expanded 25-fold during the last decade [3]. In addition, the increasing release of NPs in the environment in the future is predicted [4]. NPs may enter both aquatic and ground/soil environments through the direct use due to planned release for toxicity elimination, wastewater treatment, spillages, and deposition from the air [5, 6]. For instance, magnetite displays unique electric and magnetic properties through both Fe^{2+} and Fe^{3+} ions in its composition [7]. Iron oxide nanoparticles are used for drug delivery and in biological applications such as labelling, imaging, detection, and separation [8, 9]. According to Mohammadipour et al. [10], fertilizer with nanoiron increased Zn and Mn concentration in peace lilies, which shows less toxicity of fertilizer in contrast with chemical fertilizers. Iron oxide nanoparticle's multivalent oxidation states and abundant polymorphism result in its diverse applications such as sensors, catalysts, high-density recording medium, and clinical diagnostics [11]. According to the literature, magnetic NPs and especially iron based nanoparticles due to its high intrinsic surface reactivity have received great attention in engineering applications for water purification and soil remediation [12–14]. Fe_3O_4 NPs are extensively used in pigments, magnetic bioseparation, and lithium-ion batteries [13, 15]. A wide range of Fe_3O_4 nanoparticle applications raise the topic concerning nanomaterial safeness to the environment. According to literature, certain

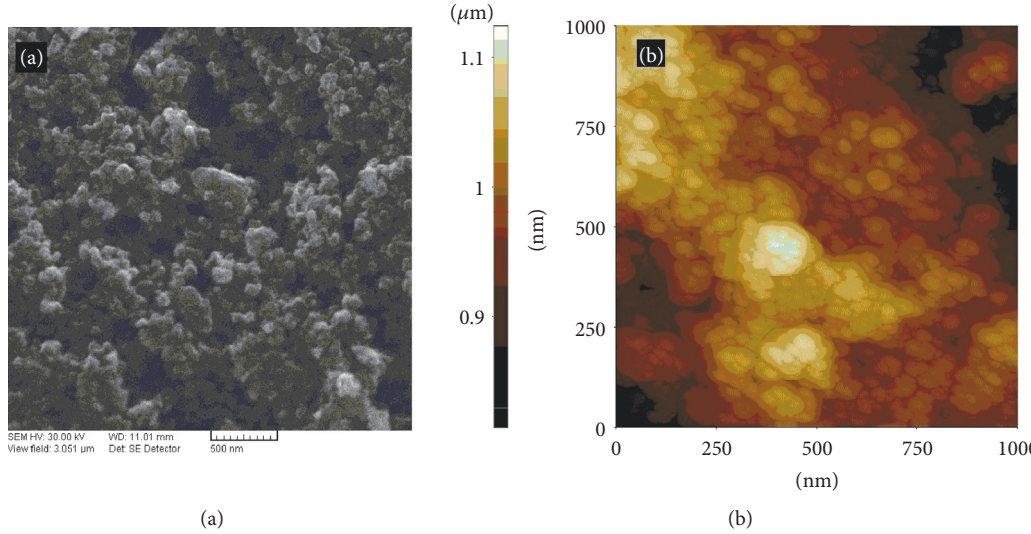

Figure 1: The images of synthesized Fe_3O_4 nanoparticles: (a) image from SEM; (b) image from AFM microscope.

nanoparticle properties lead to idea that they could possibly accumulate in living organisms, including plants, through the food chain, resulting in changes in different plant development, such as germination, nutrition, seed production, and genotoxicity [12, 16–18]. Since plants are recognized as the producers in food chain, these organisms are significant component of ecological system and it is important to know different nanoparticles influence and their interaction with plant organisms [19]. Previous investigations showed that different types, sizes, concentrations, and properties of NPs variously affect different plant species [20–24]. Iron NPs are common in the environment despite the fact that they are unstable in the presence of oxygen [25].

Flaxseed (*Linum usitatissimum* L.) is a diploid (2n) autogamous (self-fertilized) crop plant that is used by humans for the long-time period [26, 27]. Now it is predominant industrial crop cultivated in temperate climates. Flax cultivars were chosen as raw materials for manufacturing fibers and oil. Flax fiber has beneficial use in textile industry considering its fiber is long, has tensile strength, and is comprised of high quality cellulose [26]. Furthermore, flax seeds are used to produce oil, which is rich in unsaturated fatty acids, which have beneficial effects on health through presence of biologically active phytochemicals. Seeds are also utilized in animal feed, linoleum, and paints industry [26, 27]. In total, flax is extensively cultured around the world due to its commercially valuable parts as fiber, seed oil, and nutraceuticals.

Somatic embryogenesis is widely accepted by scientists as efficient method for plant micropropagation or genetic improvement which has the crucial role in plant production on the large scale [21, 28, 29]. Therefore, it is important to obtain somatic embryos on *in vitro* plant cultures [30].

According to the different investigations nanoparticles permanently affect the living organisms and may lead to DNA damage [19, 31]. Recently, RAPD method was successfully utilized for genotoxicity detection in cells of bacteria, plants, and animals due to its properties as rapid, relatively inexpensive, and effective novel biomarker assay [31–33]. RAPD markers are efficiently used for assessing genetic changes in flax genome [26].

There are several studies about different size of iron oxide NPs application and effects on different plant species. Many aspects of 4–500 nm Fe NPs influence on plants were studied, but there is no prominent information about Fe_3O_4 NPs genotoxicity in plants [34]. Therefore, the aim of present study was to detect the iron oxide NPs in flax cells and to investigate the impact of different concentrations of Fe_3O_4 nanoparticles on explants and calluses of *Linum usitatissimum* L.

2. Materials and Methods

2.1. Synthesis of Nanoparticles. Fe_3O_4 NPs were synthesized at Daugavpils University in G.Libert's Center of Innovative Microscopy, using the electrochemical method of magnetite nanoparticles synthesis [35]. The size of derived nanoparticles was 25 nm. The photograph of particles that was obtained by using the atomic-force microscope (AFM) PARK NX10 and scanning electron microscope (SEM) TESCAN VEGA LMU II can be seen in Figure 1.

2.2. Source and Micropropagation of Plant Material. For calluses formation one donor plant of *L. usitatissimum* "Vega 2" was used. *In vitro* cultures were achieved as described in Kokina et al. [21].

Two experiments with callus culture receiving were conducted. In order to obtain control samples ($n = 45$), explants were cultivated for 9 weeks onto MS medium supplemented with 1 mg/l of 2,4D (Alfa Aesar, Haverhill, Massachusetts, USA) and 1 mg/l of BAP (SERVA Feinbiochemica, Heidelberg, Germany) without NPs.

In experiment A explants ($n = 135$) were divided into three experimental groups, where the MS medium was supplemented by different concentrations of Fe_3O_4 NPs:

Table 1: Sequences of primers used for PCR reactions.

Primer ID	Sequence 5′-3′	Reference
OPF-4	5′-GAATGCGGAG-3′	Khaled et al. (2015)
CB21	5′-CAGCACTGAC-3′	Ntuli et al. (2015)
CB19	5′-GGTGCTCCGT-3′	Ntuli et al. (2015)
OPD-18	5′-GAGAGCCAAC-3′	Zargar et al. (2016)
OP-V07	5′-GAAGCCAGCC-3′	Levi and Rowland (1997)
OPD-07	5′-TTGGCACGGG-3′	Zargar et al. (2016)
OP-V15	5′-CAGTGCCGGT-3′	Levi and Rowland (1997)
OPC-08	5′-TGGACCGGTG-3′	Zargar et al. (2016)
OPE-01	5′-CCCAAGGTCC-3′	Zargar et al. (2016)
OPD-02	5′-GGACCCAACC-3′	Mohapatra and Rout (2005)
OPD-08	5′-GTGTGCCCCA-3′	Mohapatra and Rout (2005)
OPN-07	5′-CAGCCCAGAG-3′	Mohapatra and Rout (2005)
OPN-15	5′-CAGCGACTGT-3′	Mohapatra and Rout (2005)
OPA-11	5′-CAATCGCCGT-3′	Zargar et al. (2016)
OPA-10	5′-GTGATCGCAG-3′	Zargar et al. (2016)
OPA-09	5′-GGGTAACGCC-3′	Zargar et al. (2016)
OPA-07	5′-GAAACGGGTG-3′	Zargar et al. (2016)
OPA-05	5′-AGGGGTCTTG-3′	Zargar et al. (2016)
OPA-03	5′-AGTCAGCCAC-3′	Zargar et al. (2016)
OPA-02	5′-TGCCGAGCTG-3′	Zargar et al. (2016)

0.5 mg/l, 1 mg/l and 1.5 mg/l. Explants were cultivated for 9 weeks in order to obtain callus cultures till molecular biology analysis.

In experiment B explants (n = 135) were left on MS medium supplemented with 1 mg/l of 2,4D (Alfa Aesar, Haverhill, Massachusetts, USA) and 1 mg/l of BAP (SERVA Feinbiochemica, Heidelberg, Germany) without NPs for 5 weeks to accumulate callus biomass. Then obtained calluses (n = 135) were divided into control group (medium without NPs) and three experimental groups, where MS medium was supplemented by different Fe_3O_4 NPs concentrations (0.5 mg/l, 1 mg/l, and 1.5 mg/l) and was left on medium for 4 weeks.

The graphical scheme of the experiments is showed in Figure 2. The experimental treatment was repeated for three times. All calluses were cultivated in growth chamber on +24°C, 2 Lx, 16/8 h (day/night) photoperiod, and 80% humidity for five weeks. The total number of calluses was equal (n = 45) in each experimental and control group. Morphological parameters of calluses were detected by measuring the callus width, length, and regeneration type by stereo microscope Nikon SMZ 800. Confocal images of control and experimental callus thin sections were done using the microscope Eclipse Ti-E (Nikon, Japan) and blue and green laser with 405 nm and 488 nm wavelength, respectively.

2.3. Molecular Analysis. For DNA extraction calluses from both experiments were dried in silica gel for 24 h simultaneously. Total DNA from each callus was extracted from approximately 12 mg dry callus weight. Extraction was done with slight modifications using the manufacture Mini protocol: purification of total DNA from plant tissue (DNeasy Plant Mini Kit, Qiagen GmbH, Hilden, Germany) by QIAcube (Qiagen, Germany) extraction system. The final elution volume of DNA was 150 μl. DNA was quantified and qualified by spectrophotometer (NanoDrop 1000, Thermo Scientific, Waltham, USA). Stock DNA was diluted to make a working solution of 20 ng/μl for PCR analysis.

A total of 20 decamer primers were used for RAPD analysis (Table 1). PCR amplification was performed by Veriti 96 Well Thermal Cycler (Applied Biosystems, USA) thermal cycler. Each 25 μl reaction volume contained 5 μl of DNA as template, 12.5 μl of Master Mix (Taq PCR Master Mix Kit, Qiagen, Germany), 0.75 μl of $MgCl_2$ (Taq PCR Core Kit, Qiagen, Germany), 0.3 μl of primer, and 6.45 μl RNase free water. For amplification, the reaction mixtures were denatured with an initial cycle of 94°C for 1 min followed by 35 cycles of 1 min at 94°C (denaturation), 1 min 30 sec at 37°C or 50°C for OPC-08, OPD-08, and OPE-01 (annealing), and 2 min at 72°C (extension). The amplification was completed with final extension at 72°C for 10 min. As a negative control, deionized water was used instead of DNA template.

The PCR reaction products were visualized by submarine gel electrophoresis and separated on 1.3% agarose gel. The amplification reaction for each primer was conducted twice for each sample to evaluate the reproducibility of the polymorphic bands.

Data of the RAPD analysis were recorded as presence (1) or absence (0) of band products for all tested primers. The

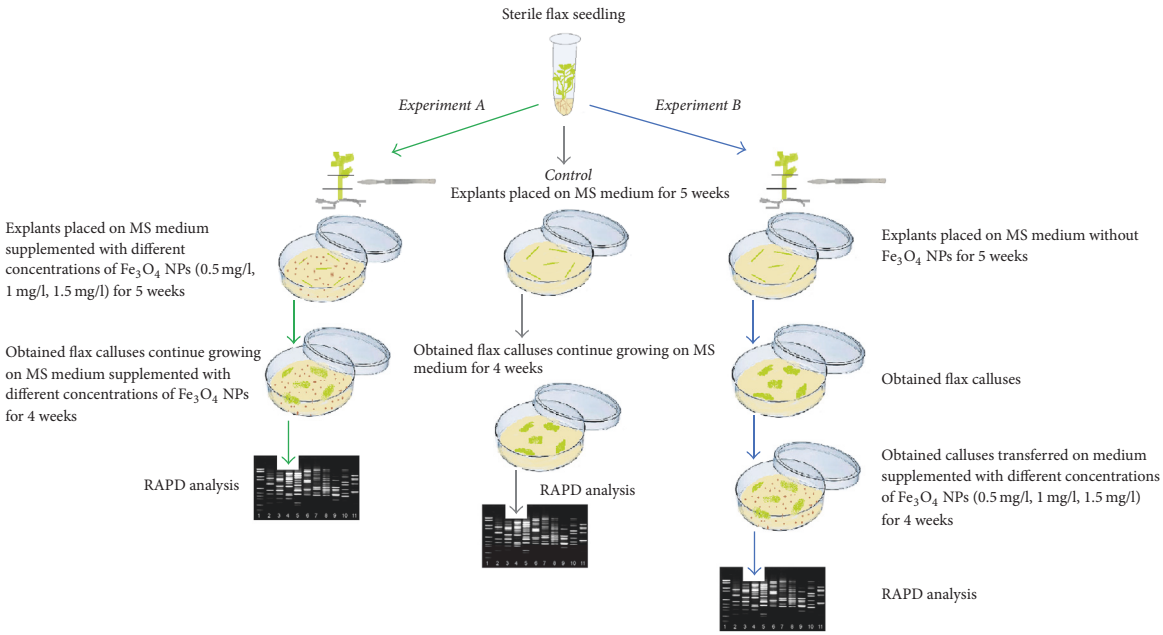

Figure 2: Scheme of plant calluses obtaining process. Flax seedlings were obtained from sterilized flax seeds and in case of experiment A were cut on explants and divided into control (without NPs) and experimental (with 0.5 mg/l, 1 mg/l, and 1.5 mg/l) groups to obtain calluses; in case of experiment B explants were grown on basal MS medium and after 5 weeks obtained calluses were transferred onto control (without NPs) and experimental (with 0.5 mg/l, 1 mg/l, and 1.5 mg/l) media for 4 weeks. Finally, DNA was extracted from both experiment calluses and RAPD analysis was carried out.

Table 2: Morphological parameters of obtained *L. usitatissimum* calluses.

Concentration of Fe_3O_4 NPs in growth medium	Number of measured calluses	Callus width, cm ± SD	Callus length, cm ± SD	Regeneration type, %
Control (without NPs)	45	0.78 ± 0.30	0.99 ± 0.40	SE* 30% R** 70%
Experiment A (explants on MS with NPs)				
0.5 mg/l	45	0.69 ± 0.24	0.84 ± 0.25	SE 100%
1 mg/l	45	0.80 ± 0.18	1.12 ± 0.34	SE 100%
1.5 mg/l	45	1.11 ± 0.26	1.4 ± 0.38	SE 100%
Experiment B (calluses on MS with NPs)				
0.5 mg/l	45	0.85 ± 0.35	1.08 ± 0.46	R 100%
1 mg/l	45	0.98 ± 0.32	1.17 ± 0.55	R 100%
1.5 mg/l	45	0.69 ± 0.30	0.73 ± 0.30	R 100%

*SE: somatic embryogenesis; **R: rhizogenesis.

obtained data of the size and weight of calluses was statistically analyzed using the t-test at two levels (0.05 and 0.01).

3. Results and Discussion

It is the first study where plant explants and calluses grown on MS medium supplemented with different concentrations of Fe_3O_4 NPs were investigated. In addition, the impact of low concentrations of iron oxide NPs onto flax callus cultures morphology and genotoxicity was explored. For this purpose, obtained control and experimental calluses were measured. Results showed that the largest calluses (width 1.11 ± 0.26 cm; length 1.40 ± 0.38 cm) were observed in experiment A on the MS medium supplemented by 1.5 mg/l of Fe_3O_4 NPs. However, the smallest calluses with average size of width 0.69 ± 0.24 cm and length 0.84 ± 0.25 cm were detected in experiment A with NPs concentration 0.5 mg/l and width 0.69 ± 0.30 cm and length 0.73 ± 0.30 cm in experiment B with NPs concentration 1.5 mg/l. These calluses were smaller than control samples (width 0.78 ± 0.30 cm; length 0.99 ± 0.40 cm).

The visually noticed true root formation (100%) occurred during experiment B, only. Nevertheless, somatic embryogenesis (100%) was noticed in experiment A in contrast to control calluses, where dominated rhizogenesis (70%) and somatic embryogenesis were detected in 30% of calluses (Table 2).

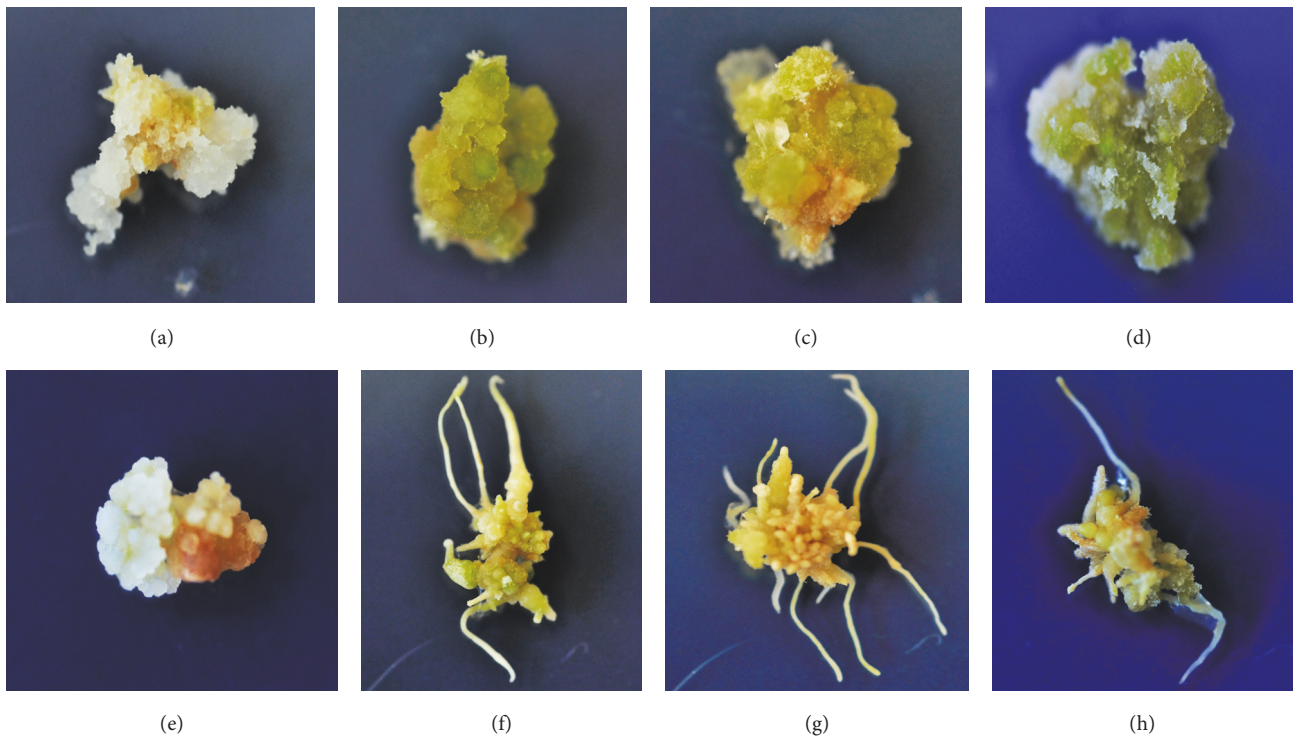

Figure 3: The morphological differences between control and treated calluses. Experiment A: (a), (b), (c), and (d). Experiment B: (e), (f), (g), and (h). Control calluses for experiment A and experiment B are (a) and (e), respectively. Calluses treated with 0.5 mg/l iron oxide NPs are (b) and (f); with 1 mg/l iron oxide NPs (c) and (g); with 1.5 mg/l iron oxide NPs (d) and (h).

Callus size varied in both experimental groups in contrast with control samples. Comparing two experiments, different amount of NPs inhibiting size of calluses can be observed. For experiment A, 0.5 mg/l NPs slightly inhibited callus size, while concentration of 1.5 mg/l most intensively stimulated callus size. In case of experiment B there was an opposite observation; respectively, the concentration of 1.5 mg/l most strongly reduced callus size.

In addition, there was difference found between color of control group calluses and two experimental groups. Control group calluses were pale and/or brown in comparison with experimental calluses that distinguish with extensively green coloration (Figure 3). Furthermore, Fe_3O_4 NPs increase chlorophyll level, root, and stems elongation [36]. Similarly, our investigation with flax callus cultures indicates treated callus coloring was bright green compared with control calluses (Figure 3).

Confocal images of control callus sections and experimental callus tissues were done. Obtained images with using blue and green laser similarly showed fluorescence inside experimental callus cells in contrast to control, which evidently show that Fe_3O_4 NPs entered experimental callus cells. Examples of acquired images are presented in Figure 4.

Nanoparticles are extremely reactive and are able to pass though the cell membrane [19, 23]. Iron oxides and their aggregates cling to the negatively charge cell surface due to electrostatic adhesion [34]. This blocking effect of NPs inhibits sufficient water uptake [37]. According to the literature, most plants accumulate heavy metals in roots [19]. Bystrzejewska-Piotrowska et al. [38] investigated translocation of Fe_3O_4 NPs in garden cress and pea. Results showed that both plants accumulated NPs with an average size 50 nm in the roots. Pumpkin plants that grew in aqueous medium supplemented with Fe_3O_4 show absorption, translocation, and accumulation of NPs in plant tissue in comparison with lima beans, where NPs uptake has not been observed [39]. Nevertheless, Marusenko et al. [15] showed that Fe magnetite NPs in the range of 22.3–67.0 nm were not taken up by *A. thaliana*. Our results reveals that 25 nm large Fe_3O_4 NPs penetrate into flax callus culture cells. It can be proved by several facts.

Firstly, Shi et al. (2015) in review which describes irradiation of Fe3O4 NPs described the photoluminescence of Fe3O4 particles with a wide size range (10 nm–5 μm) that were irradiated with 407 nm laser light (blue) and fluorescence was observed for all iron oxide particles [40]. In our experiment callus section was irradiated with 405 nm (blue) and fluorescence of callus tissue was detected.

Secondly, it is known that callus tissue cells within plant explant exhibit extensive cell division [41] and therefore there is need for active synthesis of DNA. Need for nucleic acid production promotes increased synthesis of nucleoside triphosphate (NTP) that is an early substrate for nucleic acid synthesis [42]. Increased NTP synthesis increases pH level within cells [43].

Finally, Fe3O4 NPs have high chemical stability and it is the most stable form of iron oxides [40, 44]. In addition, it is known that Fe3O4 NPs in alkaline aqueous solution are

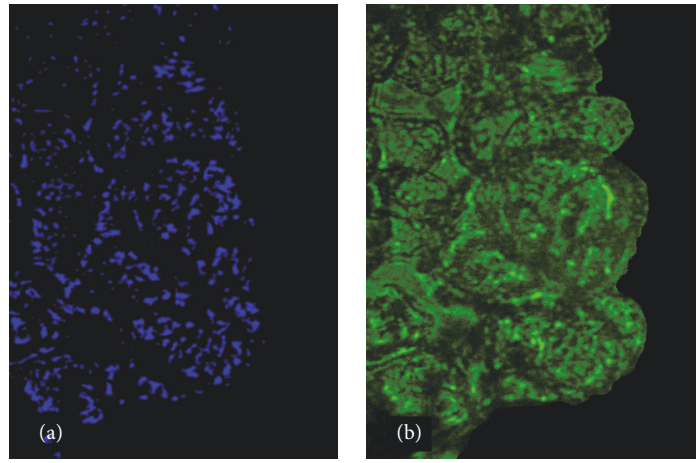

Figure 4: Examples of confocal laser scanning images of callus sections with Fe_3O_4 NPs entering cells:(a) fluorescence of Fe_3O_4 NPs in callus tissue section; (b) fluorescence of chlorophyll in callus tissue section.

sufficiently stable. Fe3O4 NPs in water and organic solvents tend to agglomerate [45]. In Figure 4(a) NPs agglomerates can be seen in the form of blue dots.

However, in Figure 4(b) fluorescence from increased chlorophyll level in experimental calluses in comparison to control samples is shown where no fluorescence was detected. It can be explained with proven fact that Fe3O4 NPs increase chlorophyll level in other plant such as oak [36] and lettuce [46]. Additionally, Vermaas et al. (2008) described chlorophyll fluorescence emission after 488 nm (green) laser irradiation [47].

In total, 20 oligonucleotide RAPD primers were utilized for detection of Fe_3O_4 NPs genotoxicity on both flax callus cultures and all primers yielded specific and stable results. Any changes in the genomic DNA amplicon pattern between control samples and samples treated with Fe_3O_4 NPs were considered to be as genotoxic changes. The selected primers generated definitive products in the range of 0.3–3.0 kb. The maximum number of bands ($n = 6$) was produced by primers CB21, OPA-11, OPD-07, OP-V15, OPA-02, OPA-03, and OPD-08, whereas the minimum number of bands ($n = 2$) was produced by primer OPC-08 (Table 3). The differences in RAPD patterns were denoted by band intensity, appearance of new bands, and loss of normal bands by contrast to the control calluses DNA. The RAPD patterns produced by some utilized primers are shown in Figure 5.

Nine of twenty primers such as OPF-4, OPD-18, OPN-15, OPA-11, OPA-05, CB19, OPV-15, OPA-02, and OPA-09 showed changes in RAPD patterns at different concentrations of Fe_3O_4 NPs treatment compared to the untreated control callus (Table 3). Differences in the DNA banding pattern were detected at different places. For instance, with OPF-04 and OPD-18 primers DNA bands disappeared at 1.5 mg/l treatment; however using OPA-11 primer 1.8 kb band was absent also at 1 mg/l treatment. In addition, in case of primer OPN-15, there was detected loss of 3.0 kb band in all samples with NPs treatment. Nevertheless, with OPA-05, CB19, OPA-02, and OPA-09 primers new bands were detected in all treated samples in comparison to control samples.

There is assumption that plant medium supplementation with metal nanoparticles causes ROS (reactive oxygen species) formation in plant tissue that leads to oxidative stress, what results in denaturation of cell structure [48] or activation of defense system in plants [17]. Wang et al. [49] showed ryegrass uptake of Fe_3O_4 NPs with a diameter of 25 nm that significantly increase oxidative stress. Also, effects of nanoparticle in plants depend on the concentration of NPs, exposure medium, and plant species [34]. According to previous data, in similar studies of NPs influence on plants, generally magnetite NPs in concentrations ranging from 30 mg/l [49] to 500 mg/l [39] were used. However, in present study relatively low concentrations of iron oxide NPs (0.5–1.5 mg/l) were investigated and DNA damage was detected.

The changes in DNA fingerprinting can be related to oxidative stress induced by metal oxide nanoparticles. RAPD technique is a fundamental tool for genotoxicity studies and is efficiently used to detect DNA changes in plants influenced by metals [50, 51]. Different stress factors including NPs can damage plant culture DNA, what can be shown as differences in band profiles [22, 50, 52]. The effect of exposition of Fe_3O_4 NPs on the DNA damage was investigated in selected model plant system under *in vitro* conditions. There was unknown Fe_3O_4 NPs influence on plant genotoxicity and statement about iron NPs passing though the cell wall was not confirmed [34]. The present study indicated changes in DNA bands induced by iron oxide nanoparticles; nevertheless, these changes could be considered as low genetic toxicity. It is interesting to note that most of new bands appeared with Fe_3O_4 NPs treatment with the exception in case of primer OPV-15, where new band appeared at 1.5 mg/l treatment only. Four primers, OPF-04, OPD-18, OPN-15, and OPA-11, showed the disappearance of bands in calluses treated with 1.5 mg/l Fe_3O_4 NPs (OPF-04 and OPD-18), with 1 mg/l NPs (OPA-11), and with 0.5 mg/l NPs (OPN-15). In addition, differences in DNA banding patterns between two experiments were not observed. Disappearance of bands possibly can be designated as DNA damage through forming of photoproducts in DNA template, or deletion of DNA fragments,

Table 3: Changes of total bands in experiments A and B.

Primer	Control		0.5 mg/l Fe_3O_4 NPs		1 mg/l Fe_3O_4 NPs		1.5 mg/l Fe_3O_4 NPs	
	A	B	A	B	A	B	A	B
OPF-4	1100	1100	1100	1100	1100	1100	-	-
	950	950	950	950	950	950	950	950
	600	600	600	600	600	600	600	600
CB21	1400	1400	1400	1400	1400	1400	1400	1400
	1300	1300	1300	1300	1300	1300	1300	1300
	1100	1100	1100	1100	1100	1100	1100	1100
	800	800	800	800	800	800	800	800
	700	700	700	700	700	700	700	700
	600	600	600	600	600	600	600	600
OPD-18	1400	1400	1400	1400	1400	1400	1400	1400
	1300	1300	1300	1300	1300	1300	-	-
	950	950	950	950	950	950	950	950
OPN-15	3000	3000	-	-	-	-	-	-
	1500	1500	1500	1500	1500	1500	1500	1500
	1300	1300	1300	1300	1300	1300	1300	1300
	300	300	300	300	300	300	300	300
OPA-07	750	750	750	750	750	750	750	750
	650	650	650	650	650	650	650	650
	550	550	550	550	550	550	550	550
OPA-11	1800	1800	1800	1800	-	-	-	-
	1600	1600	1600	1600	1600	1600	1600	1600
	1000	1000	1000	1000	1000	1000	1000	1000
	850	850	850	850	850	850	850	850
	700	700	700	700	700	700	700	700
	550	550	550	550	550	550	550	550
OPA-10	2000	2000	2000	2000	2000	2000	2000	2000
	1700	1700	1700	1700	1700	1700	1700	1700
	1000	1000	1000	1000	1000	1000	1000	1000
	500	500	500	500	500	500	500	500
OPA-05	-	-	1000	1000	1000	1000	1000	1000
	800	800	800	800	800	800	800	800
	400	400	400	400	400	400	400	400
CB19	1400	1400	1400	1400	1400	1400	1400	1400
	900	900	900	900	900	900	900	900
	-	-	450	450	450	450	450	450
OPV-07	2000	2000	2000	2000	2000	2000	2000	2000
	1700	1700	1700	1700	1700	1700	1700	1700
	1400	1400	1400	1400	1400	1400	1400	1400
	1200	1200	1200	1200	1200	1200	1200	1200
	1000	1000	1000	1000	1000	1000	1000	1000
OPD-07	2000	2000	2000	2000	2000	2000	2000	2000
	1400	1400	1400	1400	1400	1400	1400	1400
	1300	1300	1300	1300	1300	1300	1300	1300
	1200	1200	1200	1200	1200	1200	1200	1200
	800	800	800	800	800	800	800	800
	750	750	750	750	750	750	750	750

Table 3: Continued.

Primer	Control		0.5 mg/l Fe_3O_4 NPs		1 mg/l Fe_3O_4 NPs		1.5 mg/l Fe_3O_4 NPs	
	A	B	A	B	A	B	A	B
OPV-15	1700	1700	1700	1700	1700	1700	1700	1700
	-	-	-	-	-	-	1200	1200
	900	900	900	900	900	900	900	900
	700	700	700	700	700	700	700	700
	-	-	-	-	-	-	600	600
	500	500	500	500	500	500	500	500
OPD-02	1100	1100	1100	1100	1100	1100	1100	1100
	1000	1000	1000	1000	1000	1000	1000	1000
	800	800	800	800	800	800	800	800
	450	450	450	450	450	450	450	450
OPA-02	1300	1300	1300	1300	1300	1300	1300	1300
	1200	1200	1200	1200	1200	1200	1200	1200
	800	800	800	800	800	800	800	800
	-	-	700	700	700	700	700	700
	600	600	600	600	600	600	600	600
	400	400	400	400	400	400	400	400
OPA-09	1500	1500	1500	1500	1500	1500	1500	1500
	1100	1100	1100	1100	1100	1100	1100	1100
	-	-	900	900	900	900	900	900
	550	550	550	550	550	550	550	550
OPN-07	2100	2100	2100	2100	2100	2100	2100	2100
	1600	1600	1600	1600	1600	1600	1600	1600
	1000	1000	1000	1000	1000	1000	1000	1000
	750	750	750	750	750	750	750	750
	250	250	250	250	250	250	250	250
OPA-03	1700	1700	1700	1700	1700	1700	1700	1700
	1600	1600	1600	1600	1600	1600	1600	1600
	1300	1300	1300	1300	1300	1300	1300	1300
	1200	1200	1200	1200	1200	1200	1200	1200
	800	800	800	800	800	800	800	800
	300	300	300	300	300	300	300	300
OPC-08	1500	1500	1500	1500	1500	1500	1500	1500
	400	400	400	400	400	400	400	400
OPD-08	2000	2000	2000	2000	2000	2000	2000	2000
	1500	1500	1500	1500	1500	1500	1500	1500
	1000	1000	1000	1000	1000	1000	1000	1000
	900	900	900	900	900	900	900	900
	700	700	700	700	700	700	700	700
	400	400	400	400	400	400	400	400
OPE-01	800	800	800	800	800	800	800	800
	700	700	700	700	700	700	700	700
	600	600	600	600	600	600	600	600
	500	500	500	500	500	500	500	500

whereas new bands generally result in present mutations, large deletions, or homologous recombination [50, 52]. This suggests that exposition on even low Fe_3O_4 NPs concentrations (0.5 mg/l, 1 mg/l, and 1.5 mg/l) can induce damage in plant DNA level, which can result in mutation initiation.

It could confirm the above-mentioned results about chlorophyll increasing in plant cultures. Moreover, according to Mitrović et al. [53], selected stress factors that cause overproduction of ROS have been successfully utilized to improve the efficiency of *in vitro* organogenesis in plants. In case

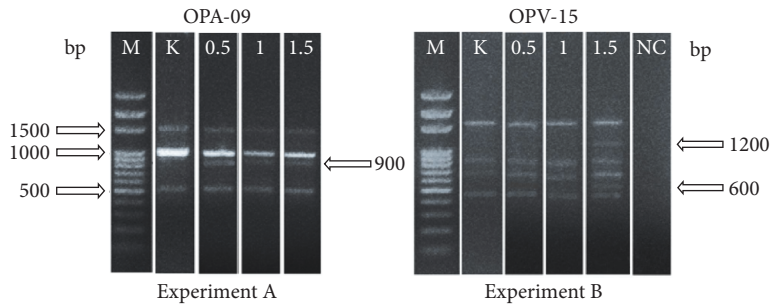

Figure 5: Examples of RAPD profile of genomic DNA isolated from *Linum usitatissimum* callus cultures in experiments A and B. M: 100 bp DNA ladder; K: control calluses (without NPs); 0.5, 1, and 1.5: experimental calluses (treated with different concentrations of Fe_3O_4 NPs, respectively, 0.5 mg/l, 1 mg/l, and 1.5 mg/l); NC: negative control.

plant is exposed to oxidative stress, to increase the cellular antioxidant resistance and adventitious root formation is the crucial case for plant survival [54]. It was found that under stress conditions *in vitro* plant cultures increase peroxidase activity and decrease H_2O_2 level. These processes induce adventitious root formation [54, 55], which were observed during experiment B, where calluses were obtained after cultivation on MS medium with NPs only on the 4-week callus stage. Translocation of calluses results in unexpected large stress inducted by probably ROS formation, what consequently led to active rhizogenesis. With reference to results of experiment A, where explants were transferred onto MS medium with addition of Fe_3O_4 it could be another process occurring. Since calluses began development on supplemented MS medium and grew longer time period (9 weeks) it is possible that there were induced adaptive processes that allows surviving in new growth conditions and formation of somatic embryos.

4. Conclusions

In conclusion, obtained results from two experiments showed that concentrations of 0.5 mg/l, 1 mg/l, and 1.5 mg/l of Fe_3O_4 NPs promote callus size and significantly increase embryogenesis level in callus cultures induced from flax explants grown on MS medium with magnetite NPs (experiment A). However, the same concentrations of Fe_3O_4 NPs essentially increase rhizogenesis level in callus cultures obtained during cultivation on MS medium with NPs only on the 4-week callus stage (experiment B). Furthermore, such low concentrations of iron oxide NPs induced genotoxicity in both experimental callus cultures.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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References

[1] S. L. Azevedo, T. Holz, J. Rodrigues et al., "A mixture toxicity approach to predict the toxicity of Ag decorated ZnO nanomaterials," *Science of the Total Environment*, vol. 579, pp. 337–344, 2017.

[2] J. E. Contreras, E. A. Rodriguez, and J. Taha-Tijerina, "Nanotechnology applications for electrical transformers—A review," *Electric Power Systems Research*, vol. 143, pp. 573–584, 2017.

[3] S. S. Patil, U. U. Shedbalkar, A. Truskewycz, B. A. Chopade, and A. S. Ball, "Nanoparticles for environmental clean-up: A review of potential risks and emerging solutions," *Environmental Technology & Innovation*, vol. 5, pp. 10–21, 2016.

[4] D. Minetto, A. Volpi Ghirardini, and G. Libralato, "Saltwater ecotoxicology of Ag, Au, CuO, TiO2, ZnO and C60 engineered nanoparticles: An overview," *Environment International*, vol. 92-93, pp. 189–201, 2016.

[5] G. Qualhato, T. L. Rocha, E. C. de Oliveira Lima et al., "Genotoxic and mutagenic assessment of iron oxide (maghemite-γ-Fe_2O_3) nanoparticle in the guppy Poecilia reticulata," *Chemosphere*, vol. 183, pp. 305–314, 2017.

[6] R. A. Villacis, J. S. Filho, B. Piña et al., "Integrated assessment of toxic effects of maghemite (γ-Fe_2O_3) nanoparticles in zebrafish," *Aquatic Toxicology*, vol. 191, pp. 219–225, 2017.

[7] M. Mahdavi, F. Namvar, M. B. Ahmad, and R. Mohamad, "Green biosynthesis and characterization of magnetic iron oxide (Fe_3O_4) nanoparticles using seaweed (*Sargassum muticum*) aqueous extract," *Molecules*, vol. 18, no. 5, pp. 5954–5964, 2013.

[8] S. W. Cao, Y. J. Zhu, M. Y. Ma, L. Li, and L. Zhang, "Hierarchically nanostructured magnetic hollow spheres of Fe_3O_4 and γ-Fe_2O_3: preparation and potential application in drug delivery," *Journal of Physical Chemistry C*, vol. 112, pp. 1851–1856, 2008.

[9] Z. Li, Q. Sun, and M. Gao, "Preparation of water-soluble magnetite nanocrystals from hydrated ferric salts in 2-pyrrolidone: mechanism leading to Fe_3O_4," *Angewandte Chemie International Edition*, vol. 44, no. 1, pp. 123–126, 2004.

[10] R. Mohammadipour, S. Sedaghat Hoor, and A. Mahboub-Khomami, "Effect of application of iron fertilizers in two methods foliar and soil applications on growth characteristics of Spathiphyllum illusion," *European Journal of Experimental Biology*, vol. 3, pp. 232–240, 2013.

[11] K. Woo, J. Hong, S. Choi et al., "Easy synthesis and magnetic properties of iron oxide nanoparticles," *Chemistry of Materials*, vol. 16, no. 14, pp. 2814–2818, 2004.

[12] M. Rizwan, S. Ali, M. F. Qayyum et al., "Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review," *Journal of Hazardous Materials*, vol. 322, pp. 2–16, 2017.

[13] S. C. N. Tang and I. M. C. Lo, "Magnetic nanoparticles: essential factors for sustainable environmental applications," *Water Research*, vol. 47, pp. 2613–2632, 2013.

[14] M. A. Usmani, I. Khan, A. H. Bhat et al., "Current trend in the application of nanoparticles for waste water treatment and purification: A review," *Current Organic Synthesis*, vol. 14, no. 2, pp. 206–226, 2017.

[15] Y. Marusenko, J. Shipp, G. A. Hamilton et al., "Bioavailability of nanoparticulate hematite to Arabidopsis thaliana," *Environmental Pollution*, vol. 174, pp. 150–156, 2013.

[16] Y. S. El-Temsah and E. J. Joner, "Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil," *Environmental Toxicology*, vol. 27, no. 1, pp. 42–49, 2012.

[17] M. N. Khan, M. Mobin, Z. K. Abbas, K. A. AlMutairi, and Z. H. Siddiqui, "Role of nanomaterials in plants under challenging environments," *Plant Physiology and Biochemistry*, vol. 110, pp. 194–209, 2017.

[18] A. Servin, W. Elmer, A. Mukherjee et al., "A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield," *Journal of Nanoparticle Research*, vol. 17, no. 2, pp. 1–21, 2015.

[19] S. C. Capaldi Arruda, A. L. Diniz Silva, R. Moretto Galazzi, R. Antunes Azevedo, and M. A. Zezzi Arruda, "Nanoparticles applied to plant science: A review," *Talanta*, vol. 131, pp. 693–705, 2015.

[20] K.-J. Dietz and S. Herth, "Plant nanotoxicology," *Trends in Plant Science*, vol. 16, no. 11, pp. 582–589, 2011.

[21] I. Kokina, E. Sļedevskis, V. Gerbreders et al., "Reaction of flax (linum usitatissimum L.) calli culture to supplement of medium by carbon nanoparticles," *Proceedings of the Latvian Academy of Sciences, Section B: Natural, Exact, and Applied Sciences*, vol. 66, no. 4-5, pp. 200–209, 2012.

[22] I. Kokina, I. Jahundoviča, I. Mickeviča et al., "The Impact of CdS Nanoparticles on Ploidy and DNA Damage of Rucola (Eruca sativa Mill.) Plants," *Journal of Nanomaterials*, vol. 2015, Article ID 470250, 7 pages, 2015.

[23] I. Kokina, I. Jahundoviča, I. Mickeviča et al., "Target Transportation of Auxin on Mesoporous Au/SiO2 Nanoparticles as a Method for Somaclonal Variation Increasing in Flax (L. usitatissimum L.)," *Journal of Nanomaterials*, vol. 2017, Article ID 7143269, 2017.

[24] A. Verma and F. Stellacci, "Effect of surface properties on nanoparticle-cell interactions," *Small*, vol. 6, no. 1, pp. 12–21, 2010.

[25] J. Tang, M. Myers, K. A. Bosnick, and L. E. Brus, "Magnetite Fe_3O_4 nanocrystals: spectroscopic observation of aqueous oxidation kinetics," *The Journal of Physical Chemistry B*, vol. 107, no. 30, pp. 7501–7506, 2003.

[26] L. M. Hall, H. Booker, R. M. P. Siloto, A. J. Jhala, and R. J. Weselake, "Flax (Linum usitatissimum L.)," *Industrial Oil Crops*, pp. 157–194, 2016.

[27] Z. Wang, N. Hobson, L. Galindo et al., "The genome of flax (*Linum usitatissimum*) assembled *de novo* from short shotgun sequence reads," *The Plant Journal*, vol. 72, no. 3, pp. 461–473, 2012.

[28] P. Asthana, M. K. Rai, and U. Jaiswal, "Somatic embryogenesis from sepal explants in Sapindus trifoliatus, a plant valuable in herbal soap industry," *Industrial Crops and Products*, vol. 100, pp. 228–235, 2017.

[29] I. Rutkowska-Krause, G. Mankowska, M. Lukaszewicz, and J. Szopa, "Regeneration of flax (Linum usitatissimum L.) plants from anther culture and somatic tissue with increased resistance to Fusarium oxysporum," *Plant Cell Reports*, vol. 22, no. 2, pp. 110–116, 2003.

[30] D. Grauda, I. Rashal, and V. Stramkale, "The use in vitro methods for obtaining flax breeding source material," in *Renewable Resources and Plant Biotechnology*, R. Kozlowski, G. E. Zaikov, and F. Pudel, Eds., pp. 127–134, Nova Publishers, New York, NY, USA, 2006.

[31] S. Salarizadeh and H. R. Kavousi, "Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of cadmium on tow iranian ecotypes of cumin (cuminum cyminum)," *Journal of Cell and Molecular Research*, vol. 7, pp. 38–46, 2015.

[32] K. Demir, M. Bakir, G. Sarikamiş, and S. Acunalp, "Genetic diversity of eggplant (Solanum melongena) germplasm from Turkey assessed by SSR and RAPD markers," *Genetics and Molecular Research*, vol. 9, no. 3, pp. 1568–1576, 2010.

[33] N. S. Kumar and G. Gurusubramanian, "Random amplified polymorphic DNA (RAPD) markers and its applications," *Science Vision*, vol. 11, pp. 116–124, 2011.

[34] N. Zuverza-Mena, D. Martínez-Fernández, W. Du et al., "Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses-A review," *Plant Physiology and Biochemistry*, vol. 110, pp. 236–264, 2017.

[35] L. Cabrera, S. Gutierrez, N. Menendez, M. P. Morales, and P. Herrasti, "Magnetite nanoparticles: electrochemical synthesis and characterization," *Electrochimica Acta*, vol. 53, no. 8, pp. 3436–3441, 2008.

[36] N. Pariona, A. I. Martínez, H. Hernandez-Flores, and R. Clark-Tapia, "Effect of magnetite nanoparticles on the germination and early growth of Quercus macdougallii," *Science of the Total Environment*, vol. 575, pp. 869–875, 2017.

[37] D. Martínez-Fernández, D. Barroso, and M. Komárek, "Root water transport of Helianthus annuus L. under iron oxide nanoparticle exposure," *Environmental Science and Pollution Research*, vol. 23, no. 2, pp. 1732–1741, 2016.

[38] G. Bystrzejewska-Piotrowska, M. Asztemborska, R. Stęborowski, J. Ryniewicz, H. Polkowska-Motrenko, and B. Danko, "Application of neutron activation for investigation of Fe_3O_4 nanoparticles accumulation by plants," *Nukleonika*, vol. 57, no. 3, pp. 427–430, 2012.

[39] H. Zhu, J. Han, J. Q. Xiao, and Y. Jin, "Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants," *Journal of Environmental Monitoring*, vol. 10, no. 6, pp. 713–717, 2008.

[40] D. Shi, M. E. Sadat, A. W. Dunn, and D. B. Mast, "Photo-fluorescent and magnetic properties of iron oxide nanoparticles for biomedical applications," *Nanoscale*, vol. 7, no. 18, pp. 8209–8232, 2015.

[41] X.-D. Wang, K. E. Nolan, R. R. Irwanto, M. B. Sheahan, and R. J. Rose, "Ontogeny of embryogenic callus in Medicago truncatula: The fate of the pluripotent and totipotent stem cells," *Annals of Botany*, vol. 107, no. 4, pp. 599–609, 2011.

[42] L. R. Engelking, "Nucleic acid and nucleotide turnover," in *Textbook of Veterinary Physiological Chemistry*, Chapter 17, pp. 98–104, Academic Press, Boston, Mass, USA, 3rd edition, 2015.

[43] E. Gout, A.-M. Boisson, S. Aubert, R. Douce, and R. Bligny, "Origin of the cytoplasmic pH changes during anaerobic stress in higher plant cells. Carbon-13 and phosphorous-31 nuclear magnetic resonance studies," *Plant Physiology*, vol. 125, no. 2, pp. 912–925, 2001.

[44] J. Xie, C. Xu, Z. Xu et al., "Linking hydrophilic macromolecules to monodisperse magnetite (Fe_3O_4) nanoparticles via trichloro-s-triazine," *Chemistry of Materials*, vol. 18, pp. 5401–5403, 2006.

[45] S. Khashan, S. Dagher, S. Al Omari et al., "Photo-thermal characteristics of water-based Fe_3O_4@SiO_2 nanofluid for solar-thermal applications," *Materials Research Express*, vol. 4, no. 5, 055701 pages, 2017.

[46] J. Trujillo-Reyes, S. Majumdar, C. E. Botez, J. R. Peralta-Videa, and J. L. Gardea-Torresdey, "Exposure studies of core-shell Fe/Fe_3O_4 and Cu/CuO NPs to lettuce (Lactuca sativa) plants: Are they a potential physiological and nutritional hazard?" *Journal of Hazardous Materials*, vol. 267, pp. 255–263, 2014.

[47] W. F. J. Vermaas, J. A. Timlin, H. D. T. Jones et al., "In vivo hyperspectral confocal fluorescence imaging to determine pigment localization and distribution in cyanobacterial cells," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 105, no. 10, pp. 4050–4055, 2008.

[48] N. Rascio and F. Navari-Izzo, "Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting?" *Journal of Plant Sciences*, vol. 180, no. 2, pp. 169–181, 2011.

[49] H. Wang, X. Kou, Z. Pei, J. Q. Xiao, X. Shan, and B. Xing, "Physiological effects of magnetite (Fe3O4) nanoparticles on perennial ryegrass (Lolium perenne L.) and pumpkin (Cucurbita mixta) plants," *Nanotoxicology*, vol. 5, no. 1, pp. 30–42, 2011.

[50] D. G. Ackova, T. Kadifkova-Panovska, K. B. Andonovska, and T. Stafilov, "Evaluation of genotoxic variations in plant model systems in a case of metal stressors," *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes*, vol. 51, no. 5, pp. 340–349, 2016.

[51] A. M. Bhaduri and M. H. Fulekar, "Biochemical and RAPD Analysis of Hibiscus rosa sinensis Induced by Heavy Metals," *Soil and Sediment Contamination: An International Journal*, vol. 24, no. 4, pp. 411–422, 2015.

[52] P. Venkatachalam, N. Jayalakshmi, N. Geetha et al., "Accumulation efficiency, genotoxicity and antioxidant defense mechanisms in medicinal plant Acalypha indica L. under lead stress," *Chemosphere*, vol. 171, pp. 544–553, 2017.

[53] A. Mitrović, D. Janošević, S. Budimir, and J. B. Pristov, "Changes in antioxidative enzymes activities during Tacitus bellus direct shoot organogenesis," *Biologia Plantarum*, vol. 56, no. 2, pp. 357–361, 2012.

[54] W. Zhang, J. Fan, Q. Tan, M. Zhao, T. Zhou, and F. Cao, "The effects of exogenous hormones on rooting process and the activities of key enzymes of Malus hupehensis stem cuttings," *PLoS ONE*, vol. 12, no. 2, Article ID e0172320, 2017.

[55] M. Libik-Konieczny, M. Kozieradzka-Kiszkurno, Ż. Michalec-Warzecha, Z. Miszalski, J. Bizan, and R. Konieczny, "Influence of anti- and prooxidants on rhizogenesis from hypocotyls of Mesembryanthemum crystallinum L. cultured in vitro," *Acta Physiologiae Plantarum*, vol. 39, no. 8, 2017.