Uptake, translocation and physiological effects of magnetic iron oxide (γ-Fe₂O₃) nanoparticles in corn (Zea mays L.)

Article in Chemosphere · September 2016
DOI: 10.1016/j.chemosphere.2016.05.083

7 authors, including:

Jing Hu
Wuhan University of Technology
3 PUBLICATIONS 3 CITATIONS
SEE PROFILE

Chuanxin Ma
University of Massachusetts Amherst
12 PUBLICATIONS 73 CITATIONS
SEE PROFILE

Jin Huang
KTH Royal Institute of Technology
267 PUBLICATIONS 3,763 CITATIONS
SEE PROFILE

All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.

Available from: Jing Hu
Retrieved on: 18 September 2016
Uptake, translocation and physiological effects of magnetic iron oxide ($\gamma$-Fe$_2$O$_3$) nanoparticles in corn (Zea mays L.)

Article in Chemosphere · September 2016
Impact Factor: 3.34 · DOI: 10.1016/j.chemosphere.2016.05.083

7 authors, including:

Chuanxin Ma
University of Massachusetts Amherst
12 PUBLICATIONS 53 CITATIONS

Jin Huang
KTH Royal Institute of Technology
260 PUBLICATIONS 3,619 CITATIONS

All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.

Available from: Chuanxin Ma
Retrieved on: 02 July 2016
Uptake, translocation and physiological effects of magnetic iron oxide ($\gamma$-Fe$_2$O$_3$) nanoparticles in corn (Zea mays L.)

Junli Li, Jing Hu, Chuanxin Ma, Yunqiang Wang, Chan Wu, Jin Huang, Baoshan Xing

School of Chemistry, Chemical Engineering and Life Science, Wuhan University of Technology, Wuhan 430070, PR China
Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA 01003, United States
Institute of Economic Crops, Hubei Academy of Agricultural Science, Wuhan 430064, PR China
School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

Abstract

Iron oxide nanoparticles ($\gamma$-Fe$_2$O$_3$ NPs) have emerged as an innovative and promising method of iron application in agricultural systems. However, the possible toxicity of $\gamma$-Fe$_2$O$_3$ NPs and its uptake and translocation require further study prior to large-scale field application. In this study, we investigated uptake and distribution of $\gamma$-Fe$_2$O$_3$ NPs in corn (Zea mays L.) and its impacts on seed germination, antioxidant enzyme activity, malondialdehyde (MDA) content, and chlorophyll content were determined. 20 mg/L of $\gamma$-Fe$_2$O$_3$ NPs significantly promoted root elongation by 11.5%, and increased germination index and vigor index by 27.2% and 39.6%, respectively. However, 50 and 100 mg/L $\gamma$-Fe$_2$O$_3$ NPs remarkably decreased root length by 13.5% and 12.5%, respectively. Additionally, evidence for $\gamma$-Fe$_2$O$_3$ NPs induced oxidative stress was exclusively found in the root. Exposures of different concentrations of NPs induced notably high levels of MDA in corn roots, and the MDA levels of corn roots treated by $\gamma$-Fe$_2$O$_3$ NPs (20 – 100 mg/L) were 5 – 7-fold higher than that observed in the control plants. Meanwhile, the chlorophyll contents were decreased by 11.6%, 39.9% and 19.6%, respectively, upon NPs treatment relative to the control group. Images from fluorescence and transmission electron microscopy (TEM) indicated that $\gamma$-Fe$_2$O$_3$ NPs could enter plant roots and migrate apoplastically from the epidermis to the endodermis and accumulate the vacuole. Furthermore, we found that NPs mostly existed around the epidermis of root and no translocation of NPs from roots to shoots was observed. Our results will be highly meaningful on understanding the fate and physiological effects of $\gamma$-Fe$_2$O$_3$ NPs in plants.

1. Introduction

With the rapid development of nanotechnology, nanoparticles (NPs) have been widely applied in various fields including environmental remediation, energy, biosensors, food industry and medicine (Casero et al., 2014; Guo et al., 2014; Lu et al., 2014; Martins et al., 2014). Currently, nanotechnology application aiming at crop protection and improvements of crop agronomic traits engenders considerable interests. Nanotechnology-based fertilizer and other agro-chemicals are promising to promote sustainable development of agriculture. Evidence exists for NPs uptake and localization of NPs inside of plant cells were reported in previous studies (Khodakovskyaya et al., 2009; Lin and Xing, 2008; Wang et al., 2012; Zhu et al., 2008). Further studies have been...
conducted to investigate impacts of NPs on plants from the aspects of morphology and physiology. For example, carbon nanotubes, gold (Au) nano-rods and titanium dioxide (TiO₂) NPs could enhance seed germination, root elongation as well as seedling growth (Khodakovskaya et al., 2009; Mondal et al., 2011; Servin et al., 2012; Wan et al., 2014; Yan et al., 2013). In contrast, other studies have reported that NPs, such as TiO₂, cerium oxide (CeO₂), zinc oxide (ZnO), γ-Fe₂O₃, could also cause damage in plants, including decreases in photosynthesis efficiency (Lei et al., 2007; Ze et al., 2011), chlorophyll degradation, total protein reduction (Krystova et al., 2013; Zhao et al., 2013), as well as micro and macro-nutrient displacement (Servin et al., 2013; Zhao et al., 2014a). Oxidative stresses caused by NPs could activate defense mechanisms to counteract nanotoxicity in plants (Morales et al., 2013; Rico et al., 2013; Servin et al., 2013). Many publications have reported the main reason of toxicity upon NPs exposure, but results were contradictory. Kim et al. (2009) suggested that the toxicity of Ag NPs is mainly due to oxidative stress and released silver ions. However, Qian et al. (2013) compared the toxicity of silver nanoparticles and silver ions on the growth of terrestrial plant model Arabidopsis thaliana and found that Ag NPs showed stronger toxicity to A. thaliana compared with Ag⁺, demonstrating that growth inhibition and cell damage of plants can be directly attributed to the nanoparticles themselves, not only the dissolved silver released from Ag NPs. And other authors reported that both silver ions and Ag NPs contribute to the toxicity (Kawata et al., 2009; Li et al., 2015; Navarro et al., 2008).

Iron plays an essential role in plants at the physiological level, especially in the process of photosynthesis. Iron deficiency in the plants directly results in chlorophyll decrease and subsequently lowers net photosynthesis efficiency. Iron deficiency is usually recognized by chlorotic or yellowed interveinal areas in new leaves, and could severely cause reduction in crop yields and even complete crop failure (Cuénot and Yi, 1994). Due to the quick conversion of Fe into plant-unavailable Fe (IIII) forms and poor mobility of Fe in phloem, soil application of inorganic Fe fertilizers to Fe-deficient soils is usually ineffective (Rengel et al., 1999). In order to address the shortcomings in the traditional iron-supplementing methods, iron oxide nanoparticles are being tested as a new iron delivery method. γ-Fe₂O₃ NPs have many advantages, such as biocompatibility, structural stability as well as magnetic properties. The mechanism of uptake and translocation of γ-Fe₂O₃ NPs and their potential toxicity require further study prior to large-scale use in agriculture. Li et al. (2013) and Ren et al. (2011) studied the physiological effects of γ-Fe₂O₃ NPs on watermelon (Citrullus lanatus) planted in quartz sand and Chinese Mung Bean (Vigna radiata L.) grown in silica sediment, respectively, and found that γ-Fe₂O₃ NPs can physiologically enhance seed germination, root growth, chlorophyll content in watermelon and mung bean, although induced oxidative stress was also evident in plants upon NPs exposure. Besides, root lengths in soybean (Glycine max (L.) Merr.) (Aldoust and Isoda, 2013) and rice (Oryza sativa L. var. Koshihikari) (Aldoust and Isoda, 2014) were increased in the presents of γ-Fe₂O₃ NPs.

However, a comprehensive study focusing on mechanisms of uptake and translocation of γ-Fe₂O₃ NPs has not yet been reported. Therefore, in our study, γ-Fe₂O₃ NPs, as an iron source, were used to investigate the effects of γ-Fe₂O₃ NPs on corn (Zea mays L.) at physiological level. Seed germination, activities of antioxidant enzymes, malondialdehyde (MDA) level and chlorophyll content were tested. In addition, we examined the uptake and translocation of γ-Fe₂O₃ NPs in corn, and analyzed the relationship between in-vivo distribution of γ-Fe₂O₃ NPs and corresponding physiological changes in plant. This study could further fill up the gaps of γ-Fe₂O₃ NPs use in agricultural crops from the aspect of nutrient supplement and figure out the mechanism of uptake and translocation of γ-Fe₂O₃ NPs in corn plants by using fluorescence labeled NPs.

2. Materials and methods

2.1. Preparation of γ-Fe₂O₃ NPs and fluorescent γ-Fe₂O₃ NPs

γ-Fe₂O₃ NPs were synthesized followed by a traditional co-precipitation method as described in Wang et al. (2015). Briefly, 2.7 g of FeCl₃·6H₂O and 1.4 g of FeCl₂·4H₂O were dissolved in 200 mL distilled water in a three-neck round-bottom flask equipped with a mechanical stirrer, reflux condenser and an inlet of nitrogen. The mixture was stirred vigorously for 15 min under nitrogen flow, followed by heating up the reaction system to 60 °C for another 30 min. A volume of 200 mL of 1 M aqueous ammonia solutions was added into the mixture, then pH was adjusted to 10–11. FeO₄ precipitation was collected via magnet and further purified with ethanol washing for five times. The collected precipitation was re-suspended in distilled water to make a suspension of 2 g/L FeO₄, which was stirred at 90 °C for 6 h with a pH of 2–3. The mixture was separated through magnetic separation, washing with absolute ethanol for three times, vacuum-dried at 60 °C for 12 h. As shown in Fig. S1A, γ-Fe₂O₃ NPs presented a spherical shape with a uniform size of 17.7 ± 3.9 nm under transmission electron microscopy (TEM).

In order to observe γ-Fe₂O₃ NPs distribution in the corn roots, fluorescent dye labeled γ-Fe₂O₃ NPs was applied (Fig. S2). The fluorescent γ-Fe₂O₃ NPs with an average diameter of 212.2 ± 29 nm were prepared as follows (Fig. S1B). The image of fluorescent γ-Fe₂O₃ NPs suspended in 1 × phosphate buffered saline (PBS) was shown in Fig. 5C. Briefly, 10 mg γ-Fe₂O₃ NPs were suspended in a mixture of 80 mL cyclohexane and 3 mL Nonidet P 40. The fluorescent dye was prepared by dissolving 3.75 mL fluorescein isothiocyanate (FITC) into 18 mL (3-aminopropyl) triethoxysilane (APTES) and 0.65 mL aqueous ammonium hydroxide solution and added into the γ-Fe₂O₃ NPs suspension drop by drop. After 24 h stirring, 0.3 mL tetraethoxysilicate (TEOS) was used to hydrolyze the bond between FITC and APTES for another 24 h. The fluorescent γ-Fe₂O₃ NPs (FITC-conjugated γ-Fe₂O₃ nanoparticles) were collected in menthol and purified by ethanol washing. The purified NPs were centrifuged at 8000 rpm for 8 min and this process was repeated 5 times. Finally, the synthesized FITC-conjugated γ-Fe₂O₃ NPs was vacuum-dried at 60 °C for 12 h. The related results of characterization of γ-Fe₂O₃ NPs and fluorescent γ-Fe₂O₃ NPs are present in supporting information, which could provide solid evidence that FITC was conjugated to γ-Fe₂O₃ NPs and FITC labeled γ-Fe₂O₃ NPs were stable and had fluorescent properties (Fig. S3–5).

2.2. Seed germination trial

Corn seeds were purchased from Hubei Academy of Agriculture Sciences, China, and thoroughly washed with reverse osmosis (RO) water prior to use. The seeds were soaked in Erlenmeyer flask containing γ-Fe₂O₃ NPs with concentrations of 0, 20, 50 and 100 mg/L for 3 h, and then germinated on moist filter paper in Petri dishes at 28 °C. Ten seeds were placed on each Petri dish; there were three replicate dishes for each NPs concentration. After 36 h, germination rate and root growth were measured.

2.3. Seeding trial

Corn seeds were soaked with deionized water in Erlenmeyer flask, and then germinated on moist filter paper in Petri dishes at
28 °C. Uniform seedlings were transferred to hydroponic system and exposed to 0, 20, 50 and 100 mg/L of γ-Fe2O3 NPs amended nutrient solution [in 1 L solution (unit: mmol L⁻¹): K₂SO₄, 0.1875; Ca(NO₃)₂, 0.5; KCl, 0.025; KH₂PO₄, 0.0625; MgSO₄·7H₂O, 0.1625; H₃BO₃, 0.25 × 10⁻³; MnSO₄·H₂O, 0.25 × 10⁻³; ZnSO₄·7H₂O, 0.25 × 10⁻³; CuSO₄·5H₂O, 0.25 × 10⁻⁴; (NH₄)₆Mo₇O₂₄·4H₂O, 1.25 × 10⁻⁶, pH 6.8]. The plants were grown in an environmentally controlled growth chamber at 28/18 °C with a 16 h/8 h light/dark cycle; the light intensity was 2000 lx. And the air was pump into the hydroponic system every 3 h for 30 min. Three replicates were applied in each treatment. Activities of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), malondialdehyde (MDA) and chlorophyll content were measured after exposure for two weeks.

2.4. Malondialdehyde (MDA) content assay

A sample of 0.35 g root or 0.25 g leaf tissues was grounded in 2.0 mL of 10% trichloroacetic acid (TCA), and the mixture was centrifuged at 1000 rpm for 10 min. A volume of 2 mL supernatant was added into 2.0 mL of 0.6% thioarbituric acid (TBA) and the mixture was boiled in water bath for 15 min and then cooled down at ambient temperature. The mixture was centrifuged at 4000 rpm for 10 min. MDA content was measured by a UV–752N spectrophotometer (Shanghai Jinke, China) at 450, 532 and 600 nm (Heath and Packer, 1968).

2.5. Antioxidant enzyme activities

The roots (0.4 g) and leaves (0.3 g) of corn were separately homogenized in 10 mL of 0.05 M phosphate buffer (pH 7.8). The mixture was centrifuged at 1000 rpm for 20 min, and stored at 4 °C until analysis.

2.5.1. Superoxide dismutase (SOD) activity assay

SOD activity was determined by the ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture consisted of 0.5 mL of supernatant, 0.5 mL of 130 mM methionine, 0.5 mL of 750 μM NBT, 0.5 mL of 100 μM EDTA-Na, 0.5 mL of 20 μM lactochrome and 3.5 mL of 0.05 M phosphate buffer (pH 7.8). The thoroughly mixed sample was illuminated for 48 h. Oven-dried root and shoots were digested with 10 mL perchloric acid (50%) in porcelain crucible for 30 min and then filtered through 0.45 μm millipore-filter. Fe content in samples was determined using Atomic Absorption Spectrometry (AAS).

2.5.2. Catalase (CAT) activity assay

CAT activity was measured by adding 0.5 mL of supernatant into 3 mL of 100 mM phosphate buffer (pH 7.0) containing 0.01% H₂O₂. CAT activity was recorded at 240 nm every 30 s for 4.5 min (Gallego et al., 1996).

2.5.3. Peroxidase (POD) activity assay

POD activity was measured based on the oxidation of guaiacol catalyzed by peroxidase (Zhang et al., 1995). Briefly, 0.5 mL of crude extract, 28 μL of 0.05 M guaiacol and 19 μL of 30% H₂O₂ were added into 100 mM phosphate buffer (pH 7.0), to form a 3 mL testing mixture. The reaction was measured at 470 nm (ΔA470) every 30 s for 4.5 min. POD activity was expressed as the change of absorbance per minute (ΔA470/min/g FW).

2.6. Chlorophyll content assay

Fresh leaves of corn (0.3 g) were ground in 10 mL of 80% acetone and then the obtained homogenates were centrifuged at 1000 rpm for 10 min. The supernatant was collected to determine the absorbance at the wavelengths of 645 and 663 nm with 80% acetone as a reference. Evaluation for total chlorophyll content was determined by the following formula:

\[ \text{Chl a} = 12.72A_{663} - 2.59A_{645} \]

\[ \text{Chl b} = 22.88A_{645} - 4.67A_{663} \]

2.7. Fluorescent observation of fluorescent γ-Fe₂O₃ NPs in plant root

Corn seedlings were cultivated in 20 mg/L FITC-conjugated γ-Fe₂O₃ NPs suspensions in a growth chamber at 28/18 °C with a 16 h/8 h light/dark cycle; the light intensity was 2000 lx, and the suspension was changed once a day. After 5 days of growth in the FITC-conjugated γ-Fe₂O₃ NPs suspensions, roots were sampled from the corn seedlings after PBS washing, followed by sample fixation in 4 vol% paraformaldehyde for 6 h. Root sections of corn were observed on a Olympus BX60 fluorescence microscope equipped with a cooled CCD camera (1410E B0, Sensys Photometrics) using MetaMorph software (Universal Imaging, version 4.5).

2.8. TEM observation of plant roots

Corn seedlings were cultivated in 20 mg/L γ-Fe₂O₃ NPs suspension for 12 days. Upon harvest, roots were sampled from the corn seedlings after PBS washing and fixed in 0.1 M PBS (pH 7.4) containing 2.5% glutaraldehyde for 4 h at 4 °C and then post-fixed in 1% osmium tetroxide for another 2 h at room temperature. Next, root samples were immersed in gradient ethanol for dehydration and acetone embedding and root samples were sectioned on Leica EM UC6 ultramicrotome for TEM observation. Ultrathin roots samples of corn were observed on a FEI Tecnai G2 20 TWIN transmission electron microscope.

2.9. Iron content in plant tissues

Root and shoot were separately harvested and dried at 70 °C for 48 h. Oven-dried root and shoots were digested with 10 mL perchloric acid (50%) in porcelain crucible for 30 min and then filtered through 0.45 μm millipore-filter. Fe content in samples was determined using Atomic Absorption Spectrometry (AAS).

2.10. Statistical analysis

Each treatment was conducted with three replicates, and the results were presented as mean ± SD (standard deviation). The statistical analysis of experimental data was verified with one-way ANOVA followed by Duncan multiple comparison in the statistical package IBM SPSS Version 22.

3. Results and discussion

3.1. Seed germination assays

The data of the seed germination test are presented in Table 1. As shown in Table 1, germination rate and germination energy were not significantly different among all treatments. However, germination index in the treatments with 20 and 50 mg/L γ-Fe₂O₃ NPs was 27.2% and 18.9% higher than the control, respectively. Vigor index in 20 mg/L γ-Fe₂O₃ NPs treatment was 39.6% higher than the
control, while that of 100 mg/L γ-Fe2O3 NPs was significantly lower (12.5%) than the control. Overall, γ-Fe2O3 NPs at lower concentration (20 mg/L) might enhance plant growth at early stage of germination.

Dose-dependent experiments were conducted to investigate the effects of γ-Fe2O3 NPs on corn seeds germination. After 2 days incubation, root length was measured every 3 h for successive 12 h. As shown in Fig. 1A, exposure to 20 mg/L γ-Fe2O3 NPs notably increased root elongation compared with the control group. At the last record, 20 mg/L of γ-Fe2O3 NPs significantly promoted root elongation by 11.5% compared with control. Phenotypic difference of root elongation at 20 mg/L was observed in Fig. 1B. Wang et al. (2012) found that multi-walled carbon nanotubes could enhance root elongation of wheat (Triticum aestivum), and they observed that root cell lengths in wheat seedlings upon exposure to 80 μg/mL α-MWNTs were increased by 1.4-fold, which suggested that cell elongation in the root system could lead to faster root growth. Kim et al. (2014) reported that nano zero valent iron enhanced root elongation by inducing OH radical-induced cell wall loosening. In order to figure out the mechanism of plants root elongation upon exposure to γ-Fe2O3 NPs, further studies need to be conducted. In addition, NPs inhibited root elongation as exposure doses increased, especially at 100 mg/L γ-Fe2O3 NPs treatment. This might be attributed to the fact that NPs in high concentration could form clusters and tend to block the pathways of nutrition uptake (Ren et al., 2011).

3.2. MDA content

MDA content is a significant parameter to assess cell membrane integrity and its content represents plant senescence and injury in the presence of pollutants (Nair et al., 2014; Zhang et al., 2007; Song et al., 2012). Fig. 2A shows MDA content in root and leaf tissues treated with different concentrations of γ-Fe2O3 NPs. Excess amounts of MDA production were evident in root tissues among all treatments relative to the control group. The MDA levels of corn roots treated by γ-Fe2O3 NPs (20–100 mg/L) were 5–7-fold higher than that observed in the control plants, indicating that oxidative stress occurred in plants. The common findings in the previous studies indicated metal-based NPs could elevate the MDA content and subsequently result in oxidative stresses in plants. Upon exposure to both 200 and 250 mg/L silver nanoparticles (Ag NPs) could significantly elevate MDA content in shoot and root of wild type (WT) Crambe abyssinica (Ma et al., 2015b). At an exposure of 1000 ppm CeO2 NPs, the MDA levels of Arabidopsis thaliana (L.) Heynh. were 4-fold higher than that observed in the control plants (Ma et al., 2013). However, in our study, γ-Fe2O3 NPs have no positive impact on MDA production in corn leaves as compared with the control. According to the result of Fe distribution (Fig. 3B), as will be discussed later), none of the γ-Fe2O3 NPs increased the level of Fe concentration in shoots, which could be the reason why no lipid peroxidation occurred in corn leaves.

3.3. Antioxidant enzyme activities

Reactive oxygen species (ROS), especially for hydrogen peroxide (H2O2) and superoxide (O2·−), induced by NPs could cause oxidative stress in plants. In order to counteract such toxicity, plant could alter activities of antioxidant enzymes such as SOD, CAT, and POD, all of which are able to scavenge ROS and further lower their toxicity. Fig. 2B–D shows the enzyme activity in corn roots and leaves treated with different concentrations of γ-Fe2O3 NPs. In plant cells, three types of SOD, containing Fe-SOD, Mn-SOD, and Cu-Zn-SOD, are able to rapidly convert O2·− to H2O2 (Ma et al., 2015b). CAT is one of the common antioxidant enzymes that convert H2O2 to H2O and O2 (Ma et al., 2015a). At an exposure of 1000 ppm CeO2 NPs, the MDA levels of Arabidopsis thaliana (L.) Heynh. were 4-fold higher than that observed in the control plants (Ma et al., 2013). However, in our study, γ-Fe2O3 NPs have no positive impact on MDA production in corn leaves as compared with the control. According to the result of Fe distribution (Fig. 3B, as will be discussed later), none of the γ-Fe2O3 NPs increased the level of Fe concentration in shoots, which could be the reason why no lipid peroxidation occurred in corn leaves.

Table 1

<table>
<thead>
<tr>
<th>Concentration of γ-Fe2O3 NPs (mg/L)</th>
<th>Germination rate (%)</th>
<th>Germination energy (%)</th>
<th>Germination index</th>
<th>Vigor index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>86.7 ± 5.7a</td>
<td>76.6 ± 5.8a</td>
<td>18.50 ± 0.21c</td>
<td>390.0 ± 4.45b</td>
</tr>
<tr>
<td>20</td>
<td>87.5 ± 5.0a</td>
<td>85.0 ± 5.8a</td>
<td>23.53 ± 0.31a</td>
<td>544.5 ± 7.19a</td>
</tr>
<tr>
<td>50</td>
<td>87.5 ± 15.0a</td>
<td>70.0 ± 18.3a</td>
<td>21.99 ± 0.95b</td>
<td>404.8 ± 17.6b</td>
</tr>
<tr>
<td>100</td>
<td>70.0 ± 14.0a</td>
<td>62.5 ± 15.0a</td>
<td>18.26 ± 1.08c</td>
<td>341.3 ± 20.3c</td>
</tr>
</tbody>
</table>

The different letters signify the value P ≤ 0.05.
increased to 100 mg/L (Fig. 2C). Our results suggest that treatment of γ-Fe₂O₃ NPs could lead to the accumulation of H₂O₂ in corn roots, which might stimulate up-regulation of CAT enzyme activity to break down the excessive amounts of H₂O₂. However, γ-Fe₂O₃ NPs treatment did not show any inhibition effect on CAT activity in the corn leaves, indicating oxidative stress did not happen in leaf tissues. Fig. 2D shows POD activity decreased in corn roots treated with 20 mg/L γ-Fe₂O₃ NPs and increased at the 100 mg/L of γ-Fe₂O₃ NP concentration. In corn leaves, POD activity was not inhibited in contrast with the control, except at the concentration of 50 mg/L. Our results of antioxidant enzyme activities suggest that γ-Fe₂O₃ NPs could activate an antioxidant defense in corn root through inducing the oxidative stress, and plant, itself, could regulate SOD, CAT and POD activities to break down excess ROS. Furthermore, comparing with the previous studies, ROS generation and antioxidant enzyme activities may vary with exposure conditions, NPs type, and plant species (Ma et al., 2015b). For example, SOD activities in ryegrass (Lolium perenne L.) and pumpkin (Cucurbita mixta) exhibited different responses upon exposure to Fe₃O₄ NPs (Wang et al., 2011). In ryegrass, SOD activity in the roots was elevated in the treatments of 30 and 100 mg/L Fe₃O₄ NPs, however, this elevation was only evident in pumpkin root treated with 30 mg/L Fe₃O₄ NPs. Similarly, the decrease in SOD activity was found in 100 mg/L Fe₃O₄ NPs treated ryegrass shoots, while Fe₃O₄ NPs had no impact on the SOD activity on pumpkin shoots.

3.4. Chlorophyll content

Chlorophyll is the critical photosynthetic pigment, chlorophyll levels can be a significant indicator of toxicity to plants (Ma et al., 2015a). As shown in Fig. 3A, for γ-Fe₂O₃ NPs treatment, the chlorophyll contents were decreased by 11.6%, 39.9% and 19.6%,
respectively, relative to the control group. Similarly, a decrease in chlorophyll content upon exposure to metal and metal oxide nanoparticles has been reported by Nair et al. (2014), Nair and Chung (2014), Barhoumi et al. (2015). Oxidative damage could happen in chloroplast within plant cells through interactions with metal-based NPs, ultimately disrupt the synthesis of chlorophyll or cause the degradation of chlorophyll in leaves (Ma et al., 2015b). However, in our study, no lipid peroxidation occurred in corn leaves, suggesting that the decrease of chlorophyll content could be an alternative mechanism. Besides, Fe content in shoots was in a low level, and shoot-to-root translocation factor (TF: Fe concentration in shoot divided Fe concentration in root) among all γ-Fe2O3 NPs treatments were significantly lower than the control (Fig. 3B–C). The inefficient translocation of iron in the plant shoots might result in the decrease in chlorophyll content.

3.5. Uptake pathway of γ-Fe2O3 NPs in corn plants

In order to figure out the pattern of uptake and distribution of γ-Fe2O3 NPs in plants, FITC-labeled NPs were applied to corn for short-term exposure. Fluorescent images of root epidermis and cross-section are shown in Fig. 4. The cyan bright dots in the image represented the FITC-conjugated γ-Fe2O3 NPs. In both Fig. 4A and B, FITC-conjugated γ-Fe2O3 NPs were observed in the epidermis. Previous studies reported that the surface of NPs could be covered by various macromolecules, and the chemical surface property and the ligand density of NPs could strongly influence the interaction between NP-bound ligands and cellular receptors (Chithrani et al., 2006; Mahmoudi and Serpooshan, 2011; Rauch et al., 2013). This could be due to the fact that macromolecules in root exudate altered physicochemical properties of the NPs surface and resulted in NPs accumulation in the root epidermis (Ghafarizan et al., 2013; Judy et al., 2012). Fig. 4C and D depict that a small fraction of FITC-conjugated γ-Fe2O3 NPs can move into endodermis and cortex of corn root, and a small portion were also located close to xylem. Most of NPs were located in the intercellular space among the cell walls of cortex tissues, which suggested that the γ-Fe2O3 NPs could penetrate corn roots and migrate between cells from the epidermis to the endodermis via the apoplastic pathway.

In order to figure out the NPs distribution at cellular level, TEM images of corn roots are shown in Fig. 5. Dark spots in Fig. 5 were observed in comparison to the control (Fig. 5A). In Fig. 5E, the size of these spots was approximately 19 nm, which was similar to the size of γ-Fe2O3 NPs, indicating the NPs could penetrate into the corn cells. As shown in Fig. 5C and D, γ-Fe2O3 NPs mainly accumulated in the vacuole. Almost all mature plant cells have large vacuoles that occupy a large part of the cell volume contained both hydrolytic enzymes and a variety of defense proteins (Hara-Nishimura and Hatsugai, 2011). Larue et al. (2012) reported the common finding that TiO2 nanoparticles (anatase, 14 nm or rutile, 22 nm) were observed in the vacuoles of wheat (Triticum aestivum spp.) roots.

3.6. Translocation of Fe from roots to shoots

As seen in Fig. 3B, the iron content in the roots exposed to 20 mg/L, 50 mg/L of γ-Fe2O3 NPs was 9.9 and 18.6 times of the control group. However, at the treatment of 100 mg/L γ-Fe2O3 NPs, Fe content in roots dramatically decreased in contrast with 50 mg/L γ-Fe2O3 NPs treatment. In Fig. 3C, notably, the TFs among all γ-Fe2O3 NPs treatments were significantly lower than the control, implying that low amounts of iron could translocate to the shoot parts (TF < 0.05 in this case). The sizes of aggregated NPs adsorbed on the root surface were larger than the size of porous space in the cell wall, only a few individual ones could move into the endodermis and cortex of corn root and were available for the upward transport. Another possible reason for the low TF could be attributed to the magnetism of γ-Fe2O3 NPs. Hong et al. (2009) concluded that γ-Fe2O3 NPs transport was influenced by a combination of electrostatic and magnetic interactions. The accumulation of large

---

Fig. 4. (A) Fluorescence image of roots epidermis, and (B, C) cross-sections of roots from corn seedlings that were treated with 20 mg/L FITC-conjugated γ-Fe2O3 NPs for 5 days; (D) is magnified view of the squared region in (C). (The cyan bright dots (NPs) in the photographs represented the FITC-conjugated γ-Fe2O3 NPs.).
amount of NPs in roots indicated that magnetically induced aggregation and subsequent straining resulted in greater retention.

Given the evidence for the absence of $\gamma$-Fe$_2$O$_3$ NPs in the NPs treated corn shoot, we further investigated $\gamma$-Fe$_2$O$_3$ NPs distribution in shoot by fluorescence microscope (Fig. 6). The corn seedlings were exposed to FITC-conjugated $\gamma$-Fe$_2$O$_3$ NPs for 5 days. The cyan fluorescence signals of the FITC-conjugated $\gamma$-Fe$_2$O$_3$ NPs were exclusively found in the root epidermis, while no presence of fluorescent signal was observed in shoot cross-section. Therefore, most $\gamma$-Fe$_2$O$_3$ NPs adhered to the surface of corn roots, and could not transport to aboveground part in spite of iron element uptake. Lack of translocation of $\gamma$-Fe$_2$O$_3$ NPs in corn plants might explain that oxidative stress and plant membrane impairment only happened in the roots rather than in the leaves. Furthermore, high accumulation of $\gamma$-Fe$_2$O$_3$ NPs in the epidermis of corn roots may have led to the shortage of the iron element in the corn leaves, and...
caused the decrease in chlorophyll content.

4. Conclusions

The comprehensive physiological effects of γ-Fe2O3 NPs on corn plants, including seed germination tests, activities of antioxidant enzymes in plant defense system, the MDA contents as well as the chlorophyll contents, have been investigated in the present work. The images from fluorescence microscope and TEM demonstrated that γ-Fe2O3 NPs were mainly accumulated in the epidermis of corn roots and migrated among cells from epidermis to endodermis by apoplastic pathway, and could further penetrate into the corn cells and localize in the vacuole. Moreover, the translocation of γ-Fe2O3 NPs in corn plants revealed that high concentrations of NPs distributed on the surface of corn roots, whereas the presence of γ-Fe2O3 NPs in the shoot was not evident. And this phenomenon could further explain the reason that oxidative stress induced by γ-Fe2O3 NPs exclusively happened in the corn roots. The inefficient translocation of iron in the plant shoots might result in the decrease in chlorophyll content. The FITC labeled γ-Fe2O3 NPs is an original attempt and our results confirmed that tracking behavior of fluorescent γ-Fe2O3 NPs in plants under fluorescence microscope could be more visualized and accurate to reflect the fate of NPs after entering root cells. There are several papers that have already reported the effects of NPs on crops over full growth cycle. For example, CeO2 NPs could affect the yield and quality of cucumber (Zhao et al., 2013, 2014b) and corn (Zhao et al., 2015), but had no impact on the final biomass and yield. Ce concentration in shoots, as well as sugar and starch contents in wheat grains (Du et al., 2015). The investigation of uptake, translocation and physiological effects of γ-Fe2O3 NPs in corn seedlings could lay a foundation for NPs potential impact in the whole life circle of corn. In conclusion, our study could help us better understand how NPs impact crop from the aspects of the physiological and biochemical levels and provide the useful information for the safe application of γ-Fe2O3 NPs in agriculture.

Acknowledgments

This work was supported by the International Science & Technology Cooperation Program of China, Ministry of Science and Technology of China (Grant No. 2014DFG52500); National Natural Science Foundation of China (Grant No. 31301735); the Fundamental Research Funds for the Central Universities (WUT: 2016B006).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.05.083.

References


