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Impact of magnetite iron oxide nanoparticles on wheat (*Triticum aestivum* L.) development: Evaluation of oxidative damage

María Florencia Iannone[a,*], María Daniela Groppa[a], María Elisa de Sousa[b], Marcela Beatriz Fernández van Raap[b], María Patricia Benavides[a]

[a] *Instituto de Química y Fisicoquímica Biológicas (IQUIFIB-CONICET), Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 Buenos Aires, Argentina*
[b] *Instituto de Física de La Plata (IFLP- CONICET), Departamento de Física, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, c.c. 67, 1900 La Plata, Argentina*

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ABSTRACT

Interest on the environmental impact of engineered nanomaterials has rapidly increased over the past years because it is expected that these materials will eventually be released into the environment. In this work, physiological effects and possible cell internalization of citric acid coated-Fe_3O_4 nanoparticles (5, 10, 15, 20 mg L^{-1}) on wheat (*Triticum aestivum* L.) plants grown five days under hydroponic conditions were evaluated. Visualization of root sections by transmission electron microscopy showed that Fe_3O_4 nanoparticles entered the root through the apoplastic route and were then detected in the root epidermal cell walls. Moreover, strong magnetic signals detected by vibrating sample magnetometry (VSM) and a huge increment in the Fe content (8,07 and 2,01 mg g^{-1} DW for NP20 and C-NP20 treatments respectively) were observed in wheat roots treated with Fe_3O_4 nanoparticles. However, no superparamagnetic signal was detected in the aerial part which indicated that magnetite nanoparticles were not translocated by vascular tissues in wheat plants in the experimental conditions of this study.

Moreover, Fe_3O_4 nanoparticles did not affect the germination rate, the chlorophyll content, and the plant growth, and they did not produce lipid peroxidation, nor alter $O_2^{\bullet-}$ or H_2O_2 accumulation respect to control plants. Furthermore, electrolyte release and cell death percentage were not modified by nanoparticle treatment. The antioxidant enzyme activities of NP treated plants significantly increased in both the root and the aerial part respect to the controls, showing a response leading to prevent oxidative damage. These preliminary results show that these Fe_3O_4 nanoparticles are not phytotoxic, suggesting that they could potentially be useful for the design of new products for agricultural use.

1. Introduction

The proliferation and release into the environment of engineered 1–100 nm diameter nanoparticles (NPs) worldwide raise important ecological and human health concerns. The increasing use of different nanoparticles for biological and industrial purposes has made necessary to go deep into the knowledge about the potential adverse effects of these nanomaterials on living organisms, since they could be transported by water and accumulate in soils (Ma et al., 2015; Hossain et al., 2015).

Considering that food materials are not only a source of nutrients but also contribute to the health of consumers, and that plants may transport NPs into the food chain, NPs could be a threat to humans and animals.

There are many reports concerning NPs uptake, translocation and toxicity in plants, but the published results are somewhat contradictory, showing variations depending on the NPs used, their size and the plant species (Arruda et al., 2015; Chichiriccò and Poma, 2015; Lin and Xing, 2007; Ma et al., 2015; Miralles et al., 2012; Ren et al., 2011 and references therein).

The first study using Fe_3O_4 NPs in plants was made by Zhu et al. (2008), who demonstrated a significant uptake of these types of NPs by pumpkin plants and their subsequent translocation and accumulation in various tissues. In soybean plants, it has been reported that iron oxide NPs affected chlorophyll content and might have influence on both biochemical and enzymatic

Abbreviations: CA, citric acid; DW, dry weight; FW, fresh weight; NPs, nanoparticles; TEM, transmission electron microscopy; VSM, vibrating sample magnetometry.

* Corresponding author.
E-mail address: mfiannone@ffyb.uba.ar (M.F. Iannone).

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efficiency in different stages of the photosynthesis reactions (Ghafariyan et al., 2013).

Although most NPs have a greater size than plant cell wall pores (3.5–5 nm) (Chichiriccò and Poma, 2015; and reference therein), it has been shown they can enter plant root cells through different mechanisms, including aquaporins (Miwa et al., 2010), endocytosis (Eggenberger et al., 2009), membrane transport systems (Miwa et al., 2010; reviewed by Gojon et al., 2009), by binding to carrier proteins or organic chemicals in the environmental media (Rico et al., 2011), creating new pores by crosslinking of components of the cell wall (Fleischer et al., 1999). According to the type of NPs, they can be accumulated in roots or be translocated to other tissues *via* xylem and phloem (Cifuentes et al., 2010). The movement of NPs between cells could occur through plasmodesmata and, inside the cells, they can be transported by endo-exocytosis or *via* apoplast and symplast (Chichiriccò and Poma, 2015; Cifuentes et al., 2010; and reference therein; Rico et al., 2011). It has been proposed that vascular tissues might play a significant role in the long-distance bulk transport of NPs (Ma et al., 2010), as occurred with CuO nanoparticles, that were transported upward by xylem and downward by phloem in maize plants (Wang et al., 2012). However, further studies are needed to understand the exact mechanisms of uptake, transport and impact of different NPs in several plant species.

Iron oxide magnetite NPs, which are generally considered to be biologically and chemically inert (Ren et al., 2011), are useful for imaging and separation techniques due to their magnetic properties. Besides, these NPs can be coated with catalysts or enzymes in order to obtain NPs with a dual function: separation and detection (Gao et al., 2007). Moreover, Fe_3O_4 NPs are increasingly used in the biomedical field (Kohler et al., 2005; Mahmoudi et al., 2011) and for environmental remediation (Liu et al., 2008; Shipley et al., 2011; Yantasee et al., 2007). In this context, the objective of this work was to determine if Fe_3O_4 NPs had toxic effects on germination and the early stages of growth of wheat plants in order to discard potential toxic effects at this essential stage, considering that these nanomaterials could be promisingly used as inoculant carriers. Taking into account that future inoculants could be formulated based on a NP concentration that did not inhibit the bacterial growth, in this work we decided to work with NP concentrations up to 20 mg L^{-1}, as has been reported by other authors that used Fe_3O_4 NPs in bacteria (Ghalamboran et al., 2009; Ghalamboran and Ramsden, 2010).

2. Materials and methods

2.1. Nanoparticles synthesis and properties

Fe_3O_4 nanoparticles were prepared by co-precipitation of ferric chloride and ferrous chloride in the presence of excess ammonia NH_4OH solution as described in de Sousa et al. (2013). The obtained magnetite cores were negatively charged by citric acid (CA) adsorption over its surfaces. Magnetite nanoparticles ~10 nm size, electrostatically stabilized by CA coating (Z-potential = −36 mV), with hydrodynamic sizes in the range~18 nm and well dispersed in aqueous solution were obtained. The electrostatic stabilization prevented aggregation during the uptake time, assuring that seeds or roots were in contact with the NPs in the whole volume of treatment solution. A colloid concentration of 20 mg ml^{-1} NPs (expressed as Fe_3O_4 mass per solution volume) was determinated within an accuracy of 2% using $K_2Cr_2O_7$ as titrant agent.

2.2. Stability assays of nanoparticles

Magnetite nanoparticles or equal millilitres of nanoparticles and Hoagland solution were electrophoresed on 2.5% horizontal agarose gel at 80 V applied voltage in order to evaluate surface charge, electrophoretic mobility and the level of purity (Morneau et al., 1999; Sahoo et al., 2005).

A turbidimetric method was used to deduce the stability in the Hoagland solution. Absorbance as a function of time was measured at 700 nm in a Hitachi U-2000 spectrophotometer (Zins et al., 1999).

2.3. Plant growth conditions and treatments

Wheat (*Triticum aestivum* L.) seeds (provided by Nidera, Argentina) were germinated on Petri dishes containing 10 ml of the treatment solution (distilled water, 5, 10, 15 or 20 mg L^{-1} of magnetite NPs in distilled water) and then placed in an incubator in the dark at 24 °C over a period of 48 h. After this time, seedlings were transferred to a hydroponic system with Hoagland (Hoagland and Arnon, 1950) solution (C) or Hoagland solution containing the NPs concentrations mentioned above. Thus, the treatments were called NP5, NP10, NP15 and NP20. When seeds were germinated in distilled water and then exposed to the NPs treatments in the hydroponic system, the treatments were called C followed by NPs concentration (C-NP): C (Hoagland solution) C-NP5, C-NP10, C-NP15 and C-NP20.

Treatment solutions were replaced daily. Plants were grown with a 16/8 h photoperiod at 26/20 °C, under fluorescent white light (photon flux density: 175 μmol m^2 s^{-1}) in a controlled environmental growth chamber. They were harvested after five days of growth and roots and aerial parts were used for analysis.

2.4. Evaluation of magnetite toxicity on wheat seed germination and seedlings growth

The germination rate of wheat seeds was recorded after the emergence of the radicle during the first 48 h of growth in distilled water or NPs treatment solutions.

To investigate the toxicity of magnetite NPs on seedling growth, a pool of 25–30 plants per treatment (C, NP or C-NP) was used to measure root or aerial part length after five days of growth on each treatment solution.

2.5. Chlorophyll content

For chlorophyll determination, 100 mg FW of wheat leaves were incubated in 5 ml of 96% ethanol at 50–60 °C until complete bleaching. Chlorophyll content was then measured spectrophotometrically at 654 nm on the ethanolic supernatant in a Hitachi U-2000 spectrophotometer, as described by Wintermans and de Mots (1965).

2.6. Iron determination

To analyze the metal concentration, seeds, roots or aerial parts were dried during 15 d at 80 °C and ground. The fine powder obtained (about 100 mg DW) was digested in a mixture of HNO_3: $HClO_4$ (3:1 v/v) at 170 °C and the metal determination was performed by flame atomic absorbance spectrometry (Perkin Elmer Analyst 300).

2.7. Oxidative stress

2.7.1. In situ $O_2^{\bullet-}$ localization

$O_2^{\bullet-}$ content was estimated using a 0.05% (w/v) solution of nitroblue tetrazolium (NBT), which reacts with $O_2^{\bullet-}$ and produces a blue precipitate of formazan. DPI (a NADPH oxidase inhibitor) was used as a control (Bolwell et al., 1998; Frahry and Schopfer, 1998).