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Quantitative Analysis of the Effect of Carbon Nanotubes on the Iron (Fe) Uptake by Corn Roots

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Abstract: Proper uptake and homeostasis of Fe by plants is critical for their growth, development, and ensuring Fe-rich plant food products for human consumption. Plants require Fe in the right amounts for life sustaining processes including respiration and photosynthesis. The objective of this study is to investigate the effect of carbon nanotubes in iron uptake by plants. Quantitative analysis of iron uptake and distribution by corn roots germinated in different media some of which are laced with Fe (II) and Fe (III) of different concentrations, and carbon nanotubes (CNT) was done using μ-PIXE (particle induced X-ray emission spectrometry). The results shows that enrichment of the germinating medium with CNT enhances Fe-uptake by corn roots.

Keywords: Iron Uptake, Iron Deficiency, Chelating, Carbon Nanotubes, μ-PIXE

1. Introduction

Iron (Fe) is one of the most essential nutrient elements for plant growth, development and yield production. It is required for a wide range of biological functions in higher plants. Among other duties, Fe forms a vital component of many enzymes like the cytochromes of the electron transport chain through reversible redox reactions cycling between Fe^{2+} and Fe^{3+}. It is also involved in the synthesis of chlorophyll as well as the maintenance of chloroplast structure and function [1]. Green plants require continuous supply of Fe since Fe does not move from the older to the newer leaves as the plant grows. Deficiency in Fe uptake thus leads to interveinal chlorosis (yellowing) in leaves which results into stunted growth and low crop yield [2, 3]. The concentrations of Fe are found to increase significantly in waterlogged areas due to low redox potentials [4]. Plants grown in such areas may take up excessive quantities of Fe which is potentially toxic as it promotes the formation of reactive-oxygen based radicals which in turn can damage cellular membranes by lipid peroxidation.

The properties of iron require plant cells to place limitations on their accumulations. Both superoxide and hydrogen peroxide produced in the cells during reduction of molecular oxygen are catalyzed by Fe^{2+} and Fe^{3+} forming highly reactive hydroxyl radicals which can damage most cellular components like DNA, proteins, lipids and sugar [5]. Since there is no evidence existing for secretory route for Fe, Fe uptake is highly regulated to achieve Fe homeostasis. In order to resist excessive uptake, wetland plants have developed a mechanism for oxidizing ferrous Fe in their rhizosphere, where as in Fe-deficient environments, plants have developed two mechanisms for improving the bio-availability of the sparingly soluble Fe compounds. These mechanisms are reduction-based and chelation-based, commonly known as *strategy I* and *Strategy II* respectively.

In a reduction based *strategy I*, non-grass plants release protons in to the rhizosphere which lowers the pH of the soil solution enhancing the solubility of Fe^{3+}. For every one unit drop in pH, it has been shown that the solubility of Fe^{3+} increases by 1000 times [2, 6, 7]. It is believed that several proton-ATPases of the AHA (Arabidopsis H^{+}-ATPase) is responsible for this process [8]. For instance, it has been found that AHA7 is un-regulated in response to Fe-deficiency

and its expression depends on Fe-deficiency induced transcription (F1T1) associating it with the proton release as part of Fe-deficiency response [9, 10]. Another *strategy I* approach involves reducing Fe^{3+} to Fe^{2+} prior to uptake to make it more soluble and absorbable. The enzyme responsible for the plasma membrane Fe^{3+} chelate reduction in the roots of *Arabidopsis* is the FRO2 [11, 12, 31]. Plants that show FRO2 over-expression are resistant to low Fe growth conditions [11]. FRO2, which belongs to an eight member gene family [13, 15, 31] is regulated both transcriptionally and post-transcriptionally in *Arabidopsis* roots. Fe (III) chelate-reductases have also been found in the roots of tomato and pea. PsFRO1 mRNA was found to accumulate in the Fe-deficient roots, suggesting its role in both Fe-uptake and transport in the roots [14].

Strategy II, which is also chelation-based, is predominantly used by grasses to acquire Fe [16]. When responding to Fe-deficiency, the grasses release Fe-binding small molecular weight compounds known as mugineic acid (MA) of the family of phytosiderophores (PS) [2, 17] which binds Fe^{3+} in the rhizosphere. Fe^{3+} is then taken up via Fe-PS transporter. Each grass produces its own sets of MAs and responds to Fe-deficiency by increasing the production and secretion of its MAs [18, 19, 20].

Nanotechnology has shown promises to improve current agricultural practices through the enhancement of management and conservation of inputs in crops as well as enhancing crop yield production [30]. In this study, quantitative analysis of Fe-uptake by corn roots germinated in different media is done. Some of the media are laced with Fe (II) and Fe (III) of different concentrations, some of which are also laced with carbon nano-tubes (CNT). The quantitative distribution of iron in different regions of the roots are analyzed and compared.

2. Sample Preparation

2.1. Corn Root Germination

Seeds of corn were germinated in vitro and in the dark in agarose gel medium. Besides the control group, there were also groups of seeds where the gel was spiked with solutions of Fe^{2+} and Fe^{3+} ions of different concentrations (1.0×10^{-3} Molar, and 3.0×10^{-4} Molar concentrations) with or without CNT. Fe (II) or Fe (III) was introduced as solutions of $FeCl_2$-$4H_2O$ and $FeCCl_3$-$6H_2O$ respectively. Once the seeds germinated, they were fixed in paraformaldehyde solution.

2.2. Corn Root Preparation for Elemental Micro-Imaging

The root radical was excised about 5 mm long and inserted in to a polyethylene tube of 5 mm diameter filled with Tissue TeK®O.C.T. medium followed by immersion into liquid nitrogen cooled isopentane for rapid freezing. The frozen blocks with samples were then mounted on the sectioning dishes in a Leica cryostat CM 3050S. The temperature of the cryo-microtome head and the chamber were set at 25°C and 20°C, respectively. The samples were sectioned with thickness of 60 µm. The frozen sections were freeze-dried, mounted freestanding on aluminum frames for micro-PIXE analysis.

3. Quantitative Elemental Micro-Imaging

The elemental micro-imaging was done using a 2-MeV focused (5 µm spot size) proton beam at the Ion Beam Modification and Analysis Laboratory (IBMAL) of the University of North Texas. We used the microprobe beamline at the NEC 9SDH tandem accelerator [25]. The average beam current of 50 pA was used to irradiate each sample with a scan area of 1 mm by 1 mm for 2 hours each. Simultaneous particle induced X-ray emission spectrometry (PIXE) and proton backscattering spectrometry (PBS) were performed using a Canberra GUL0110 HPGe X-ray detector with a resolution of 154 eV FWHM at 5.9 keV and a Canberra PIPS detector, respectively. The spectrometry system is calibrated for standard-free quantitative analysis [26]. From the PIXE data quantitative elemental images were created using the GeoPIXE software package [27] that also provides the tools to extract elemental concentrations from regions of interest. Matrix correction is based on the information on the organic matric components extracted from PBS data using SIMNRA [28]. For each sample, several sections of the roots ($n = 3 – 9$) were analyzed and the concentrations averaged for the regions of interest which included the whole root, the epidermis, the cortex, the endodermis, and the vascular tissues.

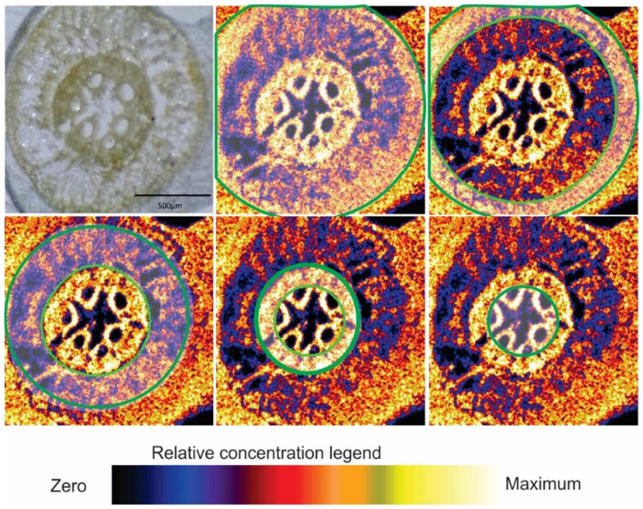

Figure 1. *Elemental maps of the corn root sample showing the regions of interest. Top row: Optical image, whole root, epidermis. Second row: Cortex, endodermis, Vascular tissues. Scan size 250 × 250 Pixels; Scan width 1000 × 1000 µm.*

4. Results

Elemental concentrations were extracted from the images in five different regions of interest: the whole root, the

epidermis, the cortex, the endodermis and the vascular tissues. The determination of elemental concentrations in different regions of the root tissue enables the comparison of the elemental concentrations and distribution in roots germinated in different media, and/or to assess the shifts in elemental depositions caused by the presence or absence of Fe (II), Fe (III), and CNT in the germinating media of each sample as described by Scheloske *et al*. [29]. µ-PIXE analysis revealed the presence of P, S, Cl, K, Ca, Ti, Cr, Fe, Cu, Zn, and As. In addition to the element of interest Fe, the concentrations of P and S recorded were the highest for each root across the samples tested. Figure 2 summarizes the results of the average P, S and Fe concentrations in ppm, measured by the µ-PIXE in the Epidermis and the endodermis of various root samples.

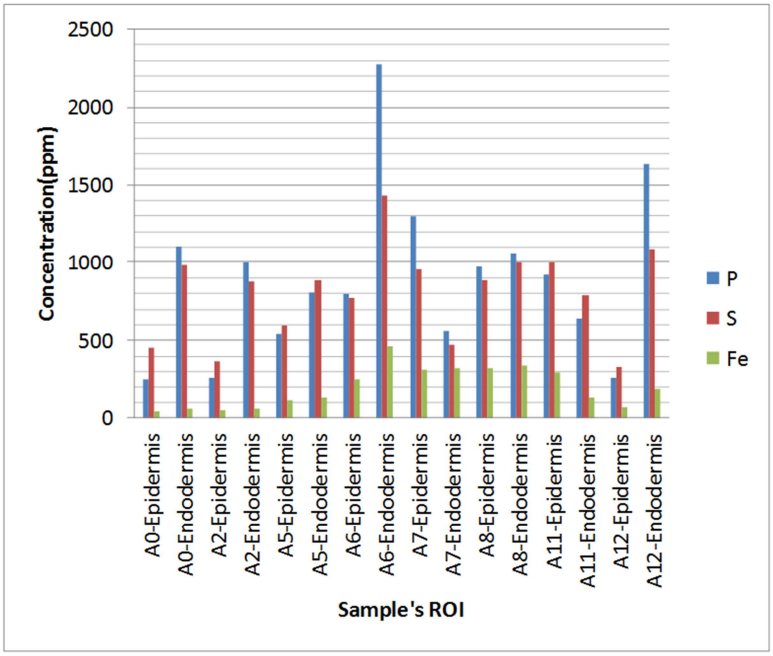

Figure 2. *µ-PIXE average elemental concentrations of P, S, and Fe for each root sample analyzed.*

It was observed that a change in the medium of germination that enhanced Fe uptake also did improve the uptake of P and S by the root samples. The sample size for each category ranged from n = 5 to n = 9.

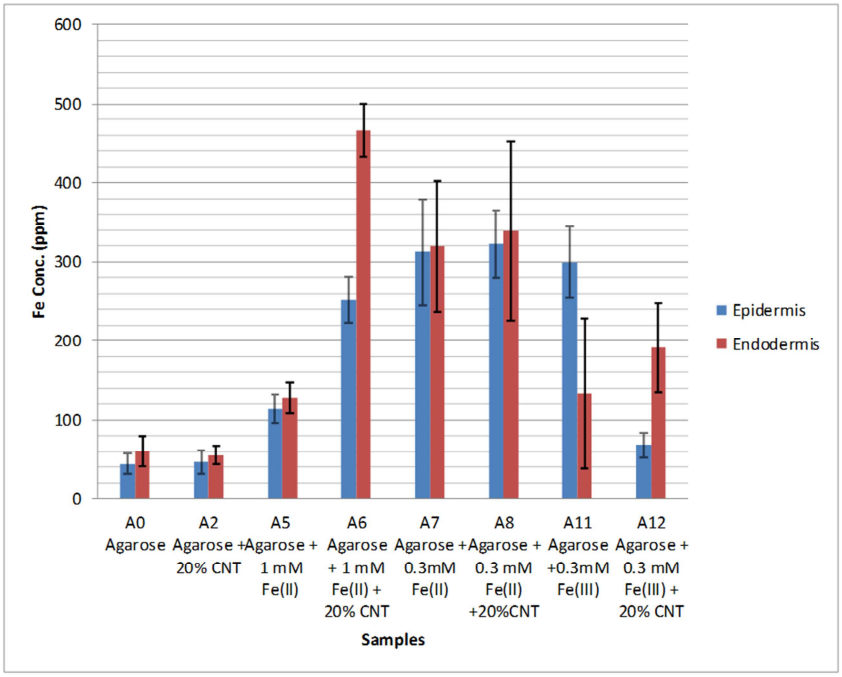

Figure 3. *µ-PIXE average elemental concentrations of Fe in the epidermis and the Endodermis regions of the root samples analyzed. Also included are the standard deviation error bars.*

5. Discussion

For sample A0 (control) the germinating medium was only agarose gel while in sample A2 (control), the seeds were germinated in agarose gel laced with 20% CNT. No significant changes in uptake of iron or the other nutrients were noticed. Simply adding CNT to the gel did not affect the uptake rates of the nutrients by the corn roots if the germinating medium is not enriched by other elements.

Analysis of sample A5 shows the effect of adding Fe (II) at a concentration of 1.0 mM to the agarose gel. Since the germinating medium was enriched with Fe (II) which is readily bio-available state, the iron uptake increased compared to the control samples A0 and A2. However, an addition of 20% CNT to the medium of 1.0 mM Fe (II), sample A6, showed much higher increase in iron uptake. The CNT in the germinating medium penetrated the seed walls activating germination and stimulated the expression of water channel genes (aquaporins) that played a critical role in the seed germination and uptake of the nutrients from the germinating medium by the exposed seeds. Water regulating aquaporins belong to a large family of major intrinsic proteins (MIPs) [24] and five sub families: plasma membrane intrinsic protein (PIP), tonoplast intrinsic protein (TIP), Nodulin-26 like proteins (NIP), small basic intrinsic protein (SIP), and X-intrinsic protein (XIP). High expressions of aquaporin have been detected in seeds exposed to CNTs which is directly correlated to the germination process.

The active role of CNT in corn seedlings germination and growth was studied by Lahiani *et al*. [21], which reported that the exposed seeds not only germinated faster but also had more developed leaves and high total shoot weight compared to non exposed seeds confirming the ability of the CNT to influence uptake of essential nutrients by the corn seeds. The level of agglomeration, the type and charge of functional groups on the seed surface as well as specific attachments play critical roles in the physiological and genetic responses of plants exposed to CNTs [22, 23, 30]. In the same study [21], it was noted that exposed seeds had an early germination and reached 100% germination compared to non-exposed seeds. When a lower concentration of 0.3 mM Fe (II) was administered to the growth medium, (sample A7), the geminating seeds may have reacted to Fe-deficiency stress mechanisms to increase the Fe uptake, compared to a medium with higher concentration of 1.0 mM Fe (II) (sample A5). This is in accordance with Liebig's law of minimum, a principle developed in agricultural sciences by Carl Sprengel (1828) and later popularized by Justus von Liebig, which states that growth of a plant is controlled not by the total amount of resources available, but by the scarcest resource. However when 20% CNT was added to the medium of 0.3 mM Fe (II), an increased uptake of iron was once again noticed which is consistent with the same explanations as given for sample A6. Due to low concentration of Fe (II), the concentrations of iron measured in different regions of interest were slightly lower than the seeds germinated in a medium with higher concentration of Fe (II).

When Fe (III) was supplied to the germinating medium, the iron uptake was remarkable less than for Fe (II). This was expected since Fe (III) is not readily bio-absorbable by plants. Since corn is in the grass family, it responds to Fe-deficiency by chelation-based *strategy II*, which involves the release of Fe-binding mugineic acid (MA) of the family of phytosiderophores (PS) [2, 17, 32]. PS which have high affinity for Fe^{3+} binds Fe^{3+} in the rhizosphere and then are taken up via Fe-PS transporter [18]. Corn secret only 2'-deoxymugineic acid (epi-DMA) in low amounts [19, 20, 33] and are therefore less tolerant to Fe-deficiency rhizosphere. This could explain lower uptake of Fe that was measured for sample A11 and A12 compared to the results for sample A7 and A8 where Fe (II) of similar concentrations were added to the germinating medium. When the medium containing Fe (III) also contained CNT, a slight increase in Fe concentration was noted in the endodermis depicting the role CNT plays in the activation of seed germination and stimulation of the expression of water channel genes (aquaporin) that plays a role in essential nutrient uptake.

6. Conclusion

Fe (II) enrichment of a medium helps to increase iron-uptake by corn roots. In the presence of CNT, the uptake is more efficient as was found in this study. A higher Fe (II) concentration in the medium does not necessarily mean a higher uptake since Fe-deficiency stress mechanisms may compensate for lower Fe availability and achieve similar Fe uptake comparatively as with higher Fe concentration in the soil. Since Fe-uptake is highly regulated by plants, the results of this study can act as a model for building up a strategy to mitigate Fe-deficiency in cornfields.

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