Hortic. Environ. Biotechnol. 57(5):462-469. 2016.
DOI 10.1007/s13580-016-0201-y

ISSN (print) : 2211-3452
ISSN (online) : 2211-3460

Research Report

Ferric-Chelate Reductase Activity is a Limiting Factor in Iron Uptake in Spinach and Kale Roots

So-Ra Lee[1,2†], Myung-Min Oh[1,2†], and Sin-Ae Park[3*]

[1]*Division of Animal, Horticultural and Food Sciences, Chungbuk National University, Cheongju 28644, Korea*
[2]*Brain Korea 21 Center for Bio-Resource Development, Chungbuk National University, Cheongju 28644, Korea*
[3]*Department of Environmental Health Science, Konkuk University, Seoul 05029, Korea*

*Corresponding author: sapark42@konkuk.ac.kr
†These authors contributed equally to this work.

Received June 29, 2016 / Revised July 24, 2016 / Accepted July 31, 2016

Abstract. Iron (Fe) is essential for many vital processes in plants, including chlorophyll biosynthesis, DNA synthesis, nitrogen reduction, and photosynthetic electron transfer. However, free Fe ions also participate in the Fenton reaction, which generates reactive oxygen species that induce oxidative stress, leading to lipid peroxidation, protein oxidation, and DNA mutation. Accordingly, plants have developed strategies to prevent roots from absorbing excess Fe. Here, we investigated Fe homeostasis in hydroponically grown spinach and kale under various Fe concentrations (5-800 μM), as well as the resulting changes in bioactive compound levels. Spinach and kale Fe contents did not increase under Fe concentrations in the nutrient solution of up to 200 μM. When spinach plants (grown in nutrient solution containing 2 μM Fe-EDTA) were transferred to 50 μM Fe-EDTA, ferric-chelate reductase activity in roots rapidly decreased within 24 hours. In kale, ferric-chelate reductase activity also significantly decreased with increasing Fe-EDTA concentration. As Fe did not accumulate in spinach plants, no significant differences in plant growth were observed. However, in kale, root growth was slightly suppressed by high Fe (100-200 μM) levels in the rhizosphere. Comparisons to other studies suggest that variations in phenolic and flavonoid contents are dependent on plant species, but overall, Fe treatment did not result in a significant increase in these compounds in spinach or kale. Our results suggest that in spinach and kale roots, the regulation of Fe contents by ferric-chelate reductase is responsible for maintaining Fe homeostasis.

***Additional key words*:** bioactive compounds, Fe-EDTA, Fe homeostasis, Fenton reaction, reactive oxygen species

Introduction

Iron (Fe) is a transition metal and an essential element in plants that is involved in chlorophyll biosynthesis (Bienfait, 1989; Bughio et al., 1997). Fe is a cofactor in the photosynthetic electron transfer chain, functioning as an electron carrier for cytochrome, ferredoxin, reductases, and oxidases. Fe is also a vital component of several enzymes related to nitrogen reduction, DNA synthesis, hormone biosynthesis, and lignin formation (Briat and Lobréaux, 1997). However, although Fe is abundant in soil, its availability is limited by low solubility under aerobic conditions (Schmidt, 2003). Consequently, Fe deficiency is a commonly occurring nutrient imbalance in plants, causing severe chlorosis that leads to declines in crop biomass production and nutritional value (Chatterjee et al., 2006; Jin et al., 2009).

Fe deficiency is also a prominent source of malnutrition in humans, inducing diseases such as anemia. According to the World Health Organization (2008), two billon people worldwide, many of whom live in developing nations, are currently suffering from Fe deficiency. A nutritional survey carried out by the Korean Ministry of Health and Welfare (2007) revealed that most (75.8%) daily food intake comprises plant-based foods, whereas animal-based foods account for only 24.2%. Since the non-heme form of Fe in vegetables and plant-based foods has a very low absorption rate in the human body, nutritionists recommend the consumption of heme Fe from animal-based products to address Fe deficiency. Unfortunately, recent studies have reported that heme Fe intake is closely associated with several diseases such as stroke, colorectal cancer, and ovulatory infertility; consequently, there is an emerging need for plant-based foods as a source of Fe (Chavarro et al., 2006; Bastide et al., 2011; Kaluza et al., 2013). To treat Fe deficiency anemia without causing a

host of other health problems, we must improve the absorption rate of non-heme Fe and enhance the Fe content in vegetables.

In plants, two types of strategies exist for Fe absorption through roots. Dicots and non-graminaceous monocots are Strategy I plants that release protons into the rhizosphere to activate membrane-bound Fe(III)-chelate reductases, which reduces Fe(III) to the absorbable Fe(II); Fe(II) uptake into root cells is performed by the Fe transporter, iron-regulated transporter1 (IRT1) (Waters et al., 2002). Graminaceous monocots are Strategy II plants that synthesize and release the Fe(III)-binding compounds phytosiderophores (PS) into the surrounding soil; uptake of the resulting Fe(III)-PS complex is performed through the complex transporter, yellow stripe1 (YS1). Both strategies are activated under conditions of Fe deficiency (Grusak et al., 1990; Montgomery et al., 2015), indicating that even if Fe concentrations are high in the rhizosphere, plants may absorb and use only a limited amount of Fe.

Typically, in well-aerated soil, Fe is oxidized to an insoluble ferric form [Fe(III)], whereas under waterlogged or wetland conditions, Fe is reduced and solubilized to the ferrous form [Fe(II)] that can be mobilized in plants (Martin, 1968). However, plants may absorb Fe in excess, inducing oxidative stress (Hendry and Brocklebank, 1985) through the production of reactive oxygen species (ROS) under the Fenton reaction. Plant cell damage can occur via lipid peroxidation, protein oxidation, and DNA mutation. To prevent Fe cytotoxicity, plants have a highly controlled Fe-absorption system that maintains the Fe content at an optimum level for growth and development (Briat and Lebrun, 1999; Schmidt, 2003). Despite these mechanisms, increasing the Fe content within a certain range may generate enough ROS to increase the levels of bioactive compounds, including antioxidants, without substantially harming the plant (Alscher et al., 1997; Park et al., 2014).

Ultimately, to mitigate Fe deficiency in humans, research should focus on Fe fortification of plant-based foods by taking advantage of Fe homeostasis mechanisms in plants. Moreover, it is important to analyze the effect of Fe uptake on bioactive compounds produced in plants. Studies on Fe homeostasis have mainly been conducted in *Arabidopsis* or cereal crops, but such studies in vegetables are limited (Schmidt, 2003). Therefore, in the current study, we grew spinach and kale hydroponically under various Fe concentrations to explore Fe homeostasis and to observe changes in the levels of bioactive compounds.

Materials and Methods

Plant Materials and Growth Conditions

Spinach (*Spinacia oleracea* cv. Melody) and kale (*Brassica oleracea* var. *acephala* cv. TBC) were used in this study. Seeds of both crops were sown on seed growth pouches (DIK-710A, Daiki, Saitama, Japan) and germinated for 7 days (spinach) and 18 days (kale). Seedlings were transferred to a deep flow technique system in a growth chamber (DS-51GLP, Dasol Scientific, Hwaseong, Korea) for a 4-week cultivation period under 20/15°C day/night air temperatures and 60% relative humidity. Fluorescent lamps were used as an artificial lighting source (photosynthetic photon flux [PPF] 230 and 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for spinach and kale, respectively; 12-hour light period). To minimize light-distribution imbalance, the plants were systematically rotated every other day.

Fe Supply

During the 4-weeks cultivation period, the spinach and kale plants were supplied with half-strength Hoagland's nutrient solution containing Fe (II)-ethylenediamine-tetraacetic acid (EDTA) at 5, 25, 50, 75, 100, 150, and 200 μM. The nutrient solution was maintained at an electrical conductivity (EC) of 1.14 $dS \cdot m^{-1}$ and pH 5.8. To keep the pH steady, 2 mM MES buffer (Sigma-Aldrich, St. Louis, MO, USA) was added to the nutrient solution.

An additional experiment was performed to determine the dynamic changes in the activity of ferric-chelate reductase, an enzyme related to root Fe uptake. Spinach plants grown in nutrient solution containing 2 μM Fe-EDTA for two weeks were transferred to nutrient solution containing 50 μM Fe-EDTA. Kale plants were cultivated separately in nutrient solution containing Fe-EDTA at 25, 50, 100, 200, 400, and 800 μM for 4 weeks; enzyme activity in roots was monitored during this time.

Growth Characteristics

Seven spinach and nine kale plants per treatment were collected on the fourth week after transplanting. The plants were weighed using an electronic scale (Si-234, Denver Instrument, Bohemia, New York, USA) after being separated into shoot and root fractions. Both plant parts were oven-dried (FS-420, Advantec Co., Bunkyo-ku, Tokyo, Japan) at 70°C for 72 hours and weighed to determine dry weight.

Fe Content

Spinach and kale shoots were treated with a wet digestion method using nitric acid (HNO_3), as described by Havlin and Soltanpour (1980) with minor modifications. Individual dried spinach leaves were separated into three groups according to position (low, middle, high), and approximately 0.2 g leaf tissue from each group was used to determine Fe content in spinach. Approximately 1 g of dried kale shoots was used. The samples were digested in 15 mL of 70% HNO_3 at 125°C on a heating block (OD-98-002P, Odlab, Seoul, Korea) for

1.5 hours. To stimulate digestion, the solution was combined with 7.5 mL of H_2O_2, heated for 1 hour, combined with an additional 7.5 mL of H_2O_2, and heated to 200°C for 2 hours. The solution was cooled, mixed with 45 mL of 2% HNO_3, and resuspended for 3 hours. The solution weight was then increased to approximately 90 g using triple-distilled water and filtered through quantitative filter paper (Advantec Co., Tokyo, Japan). The Fe concentration of the solution was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 7300 DV, Perkin Elmer, Waltham, MA, USA).

Ferric-Chelate Reductase Activity

Ferric-chelate reductase activity in the whole root systems of spinach and kale was determined by tracking Fe(II) chelation to bathophenanthrolinedisulfonic acid (BPDS) (Sigma-Aldrich) according to Grusak (1995). Spinach and kale roots were cut with a sterilized scalpel and placed into tubes containing 50 mL of Hoagland's nutrient solution with added air bubbles. The nutrient solution was immediately replaced with 50 mL of assay solution (Hoagland's solution, 1 mM MES buffer, 100 μM Fe-EDTA, and 100 μM Na_2-BPDS) (Sigma-Aldrich), and the roots were allowed to react for 20 minutes with the added air bubbles. The solution turned red after the formation of Fe(II)-BPDS; the absorbance was recorded at 535 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The roots were extracted from the solution, wiped dry, and weighed. Enzyme activity was calculated with the following equation:

$$\text{Reductase activity} = \frac{(\text{Avg } A_{535}\ \text{cm}^{-1}/0.02214\ \mu\text{M}^{-1}\text{cm}^{-1}) * (\text{Volume of soluble assay solution (L)})}{\text{Time (h)} * \text{FW (fresh weight) of root (g)}} = \mu\text{mol/g FW} * \text{h}$$

Bioactive Compounds

***Total phenolic content and antioxidant capacity*:** The total phenolic content and antioxidant capacity of spinach and kale leaves were analyzed using Folin-Ciocalteu (Ainsworth and Gillespie, 2007) and Trolox (Campos and Lissi, 1996) reagents, respectively. The fourth leaf from the base of each spinach and kale plant was collected, and a 0.2-g tissue samples were stored at -70°C (IE2386D, Thermo Fisher Scientific, Pittsburgh, USA) for subsequent analysis. Sample extraction and reaction procedures were performed as described by Lee et al. (2014), and the absorbance of the final reaction solution was determined at 765 nm (for total phenolic content) and 730 nm (for antioxidant capacity) using a UV-Vis spectrophotometer. Total phenolic content was expressed as the amount of gallic acid (mg) (Acros Organics, Belgium) per 1 g of fresh weight. Antioxidant capacity was expressed as Trolox concentration (mM) (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxyl acid; Sigma-Aldrich).

***Total flavonoid content*:** Total flavonoid content in kale leaves was determined according to Zhishen et al. (1999), with slight modifications. Approximately 0.2 g of kale leaf tissue was weighed and ground in a mortar in liquid nitrogen; 70% ethanol was adjusted to pH 3.2 using formic acid and added to the ground leaf tissue for extraction. The extracts were transferred into micro-tubes, incubated in darkness at 4°C for 12 hours, and centrifuged at 2000 × *g* for 2 minutes. The supernatant was mixed with 1.25 mL of distilled water and 75 μL of 5% $NaNO_2$. After 5 minutes, 150 μL of 10% $AlCl_3$ was added to the sample; after an additional 6 minutes, 500 μL of 1 M NaOH and 275 μL of distilled water were added to the sample. The solution was mixed well and incubated for 5 minutes, after which its absorbance was measured in a spectrometer against a blank (70% ethanol) at 510 nm. Total flavonoid content in kale leaf was expressed as the amount of (+)-catechin (mg) per 1 g of fresh weight.

***Individual phenolic compounds*:** Samples were extracted and separated according to Bergquist et al. (2005) with slight modifications. Approximately 0.5 g of frozen spinach leaf tissue was ground in a mortar in liquid nitrogen. The sample was extracted with 10 mL of 40% methanol, shaken for 20 hours at 150 rpm and 4°C, and centrifuged at 10,000 × *g* for 10 minutes. The supernatant was stored at -80°C until analysis using high performance liquid chromatography (HPLC) (Dionex IC3000, Thermo Fisher Scientific). Using a C18 column (5.0 μm, 250 × 4.6 mm; 201TP54; Phenomenex, Torrance, CA) fitted with a guard column (7.0 μm, 4 × 3.0 mm) compartment, all samples were analyzed for five phenolic compounds: caffeic acid, ferulic acid, *p*-coumaric acid, rutin, and quercetin. The mobile phase was a gradient of (A) water/methanol/formic acid (69:30:1 v/v) and (B) methanol, as follows: 0-18 min, 15-45% eluent B; 18-23 min 45-100% B; 23-27 min 100% B. The flow rate was 0.7 mL/min. The absorbance was recorded at 340 nm using a diode array detector. The sample injection volume was 10 μL. The five phenolic compounds were used as standard materials (Sigma-Aldrich).

Statistical Analysis

Spinach and kale growth were measured by weighing seven and nine plants per treatment, respectively. Five plants per treatment were used to determine Fe and bioactive compound contents in both plants. Ferric-chelate reductase activity was determined using three (spinach) and five (kale) plants per treatment. Data were analyzed using one-way analysis of variance (ANOVA) in SAS (Statistical Analysis System,

9.2 Version, SAS Institute Inc., Cary, NC, USA). Treatment means were compared using the Duncan's multiple range test. Significance was set at $p = 0.05$.

Results

Spinach and Kale Growth

The addition of various concentrations of Fe-EDTA in the nutrient solution did not have a meaningful impact on spinach growth after 4 weeks of treatment (Fig. 1). In general, no significant differences were detected for kale, although both the fresh and dry weights of the shoot tended to decrease slightly under high Fe-EDTA concentrations. However, at high Fe-EDTA concentrations (over 100 μM), both the fresh and dry weights of the root significantly decreased (Table 1). In particular, the root fresh weight and dry weight under 200 μM treatment were lower than those under the 25 μM control treatment by 51% and 50%, respectively.

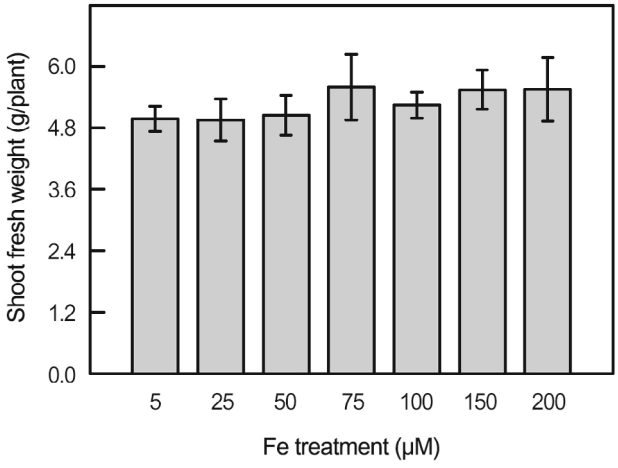

Fig. 1. Shoot fresh weight of spinach cultured in nutrient solution containing various concentrations of Fe-EDTA for 4 weeks (n = 7). Error bars indicate standard errors.

Spinach and Kale Fe Homeostasis

Spinach leaf Fe contents were highest under 50 and 150 μM treatment, but the results were not significantly different under any of the treatments (25–200 μM Fe) (Fig. 2A). Fe contents also increased with higher leaf position: leaves at the middle and highest positions contained more Fe than those at the lower position by approximately 14% and 42%, respectively. By contrast, the Fe contents in kale shoots did not significantly differ across treatments (25–200 μM Fe) (Fig. 2B).

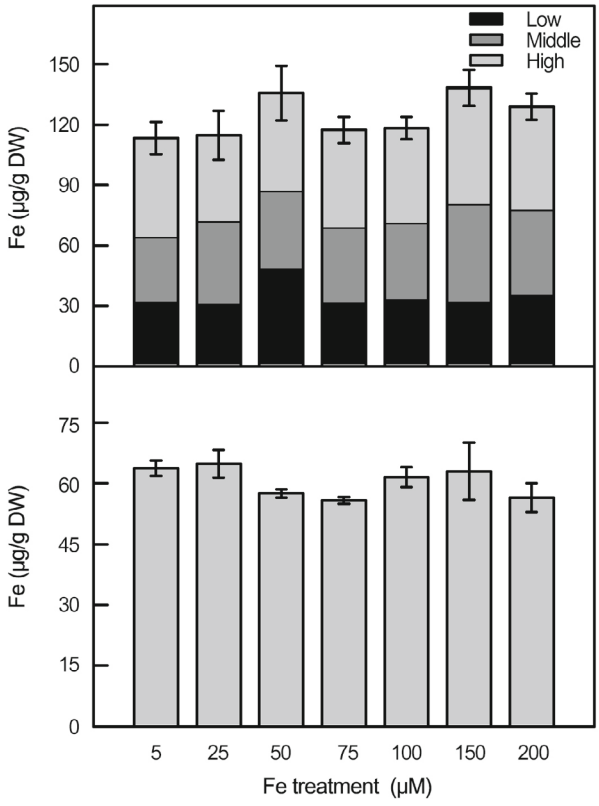

Fig. 2. Fe content in spinach and kale cultured in nutrient solution containing various concentrations of Fe-EDTA for 4 weeks (n = 5). Error bars indicate standard errors.

Table 1. Growth of kale cultured in nutrient solution containing various concentrations of Fe-EDTA for 4 weeks

Fe treatment (μM)	Shoot		Root	
	Fresh weight (g/plant)	Dry weight (g/plant)	Fresh weight (g/plant)	Dry weight (g/plant)
5	7.86 ± 0.62[z]	0.57 ± 0.05	0.72 ± 0.08 ab[y]	0.05 ± 0.01 ab
25	8.49 ± 0.49	0.60 ± 0.04	0.77 ± 0.04 a	0.06 ± 0.00 a
50	7.02 ± 0.31	0.50 ± 0.03	0.56 ± 0.06 abc	0.04 ± 0.00 bc
75	8.68 ± 0.90	0.63 ± 0.07	0.71 ± 0.09 ab	0.06 ± 0.01 a
100	7.61 ± 0.68	0.55 ± 0.05	0.49 ± 0.08 bc	0.04 ± 0.00 c
150	7.17 ± 0.87	0.52 ± 0.06	0.58 ± 0.09 abc	0.04 ± 0.01 abc
200	6.27 ± 0.56	0.46 ± 0.04	0.37 ± 0.06 c	0.03 ± 0.00 c

[z]Data are shown as mean ± standard error (n = 9).
[y]Mean separation within columns using Duncan's multiple range test at $p = 0.05$.

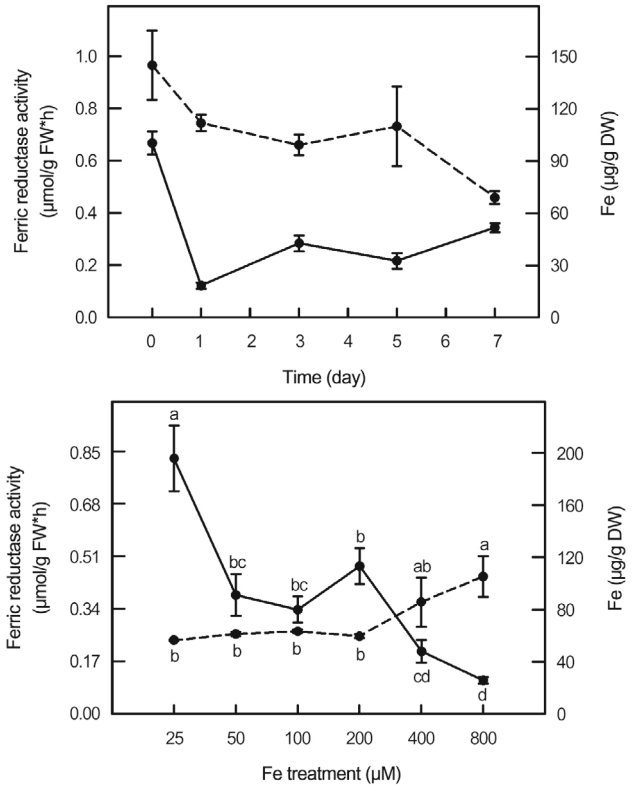

Fig. 3. Ferric-chelate reductase activity (solid line) and Fe content (dotted line) in spinach (n = 3) transferred from 2 weeks of culture in 2 μM of Fe-EDTA to 50 μM of Fe-EDTA (A) and in kale (n = 5) cultured in nutrient solution containing 25, 50, 100, 200, 400, and 800 μM of Fe-EDTA for 4 weeks (B). Different lowercase letters indicate significant differences at p = 0.05. Error bars indicate standard errors.

When spinach was subjected to an increase in Fe-EDTA after 2 weeks of cultivation in 2 μM Fe-EDTA, the subsequent week saw a drastic decrease in ferric-chelate reductase activity on the first day, which persisted on days 3, 5, and 7 (Fig. 3A). For kale, after cultivation at various Fe-EDTA concentrations for 4 weeks, ferric-chelate reductase activity declined significantly as Fe concentration increased (Fig. 3B). Moreover, under all treatment conditions except 200 μM Fe, the ferric-chelate reductase activity and Fe-EDTA concentration had a linear negative correlation in kale. The Fe content in kale shoots increased at 400 and 800 μM Fe-EDTA treatment, with the Fe uptake rate significantly higher under 800 Fe μM Fe-EDTA treatment compared to treatments below 400 μM (Fig. 3B).

Bioactive Compounds in Spinach and Kale

Fe levels in the nutrient solution affected bioactive compound levels in spinach (Fig. 4). The total phenolic content was highest under 50 μM Fe treatment and tended to decrease gradually with increasing Fe concentration, with the lowest content (significantly lower than the control) detected under 200 μM Fe treatment (Fig. 4A). HPLC analysis revealed that for all five phenolic compounds (caffeic acid, ferulic acid, *p*-coumaric acid, rutin, and quercetin), the contents tended to be lower under 50 and 150 μM Fe treatment compared to 5 μM Fe treatment. In particular, Fe levels had pronounced effects on caffeic acid and ferulic acid contents (Fig. 4B).

In kale, no significant difference in total phenolic content or antioxidant capacity was observed across treatments, but both values tended to increase with increasing Fe level, with a peak detected at 800 μM Fe, followed by a decline. By contrast, no general trend or significant difference in total flavonoid content was detected across Fe treatments (Fig. 5).

Discussion

Spinach and Kale Growth

Minerals are among the most important nutrients in leafy vegetables. Spinach and kale are leafy vegetables with high

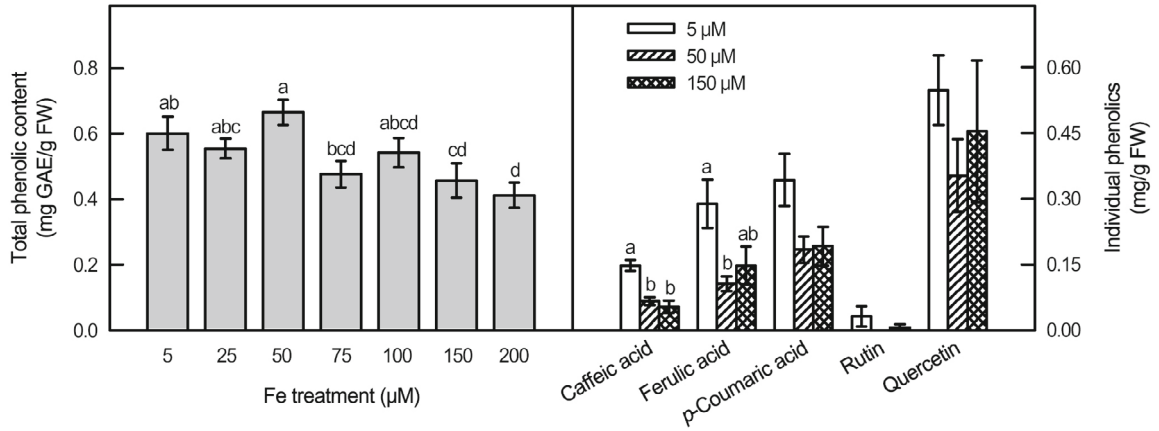

Fig. 4. Total phenolic content (A) and individual phenolic content (B) in spinach leaves cultured in nutrient solution containing various concentrations of Fe-EDTA for 4 weeks. Different lowercase letters indicate significant differences at p = 0.05 (n = 5 for total phenolic content, n = 3 for individual phenolic content). Error bars indicate standard errors.

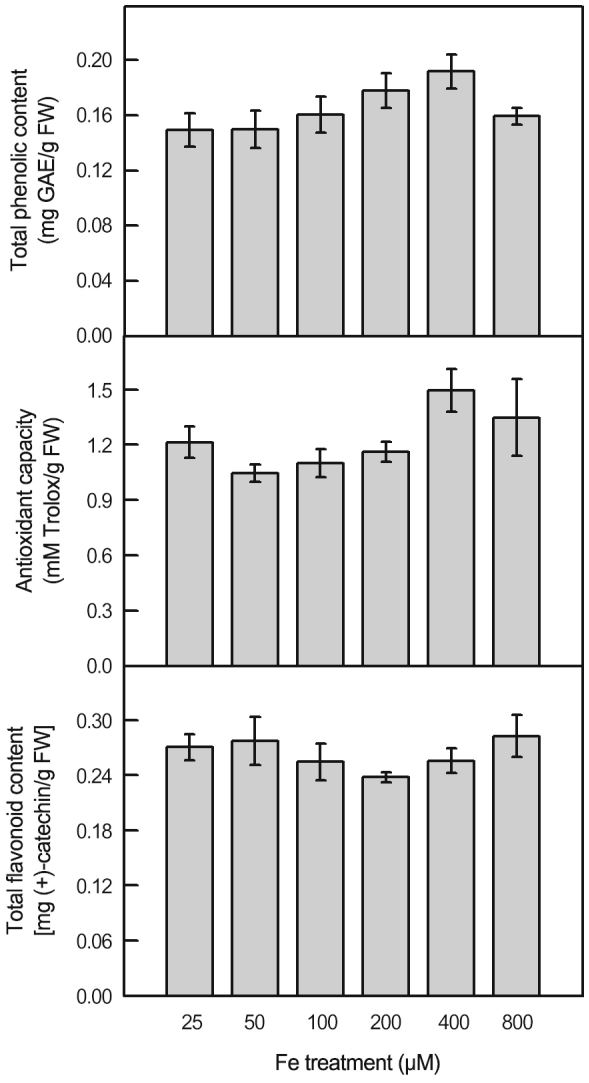

Fig. 5. Total phenolic content (A), antioxidant capacity (B), and total flavonoid content (C) in kale leaves cultured in nutrient solution containing various concentrations of Fe-EDTA for 4 weeks (n = 5). Error bars indicate standard errors.

contents of minerals, including K, Mg, and Ca. In particular, Fe contents are high in both vegetables (Mills and Jones, 1996; Bhattacharjee et al., 1998).

In spinach, we observed no changes in shoot growth regardless of Fe concentration during cultivation. In general, both a lack and an excess of plant Fe accumulation cause declines in plant growth due to either malnutrition or oxidative stress (Briat and Lebrun, 1999). We found that even when Fe-EDTA concentrations in the nutrient solution were far above control levels, Fe uptake in spinach did not significantly increase. This observation likely explains why spinach growth did not significantly differ across treatments.

Similar to spinach, Fe treatments did not strongly affect kale shoot growth. However, kale root growth tended to decrease under Fe concentrations over 100 μM. Our results are partially similar to findings for other plants. In *Phragmites australis* treated with 0-50 $mg \cdot L^{-1}$ of $FeSO_4 \cdot 7H_2O$, treatments over 2 $mg \cdot L^{-1}$ caused a decline in root growth rate and shoot dry weight (Batty and Younger, 2003). They suggested that high Fe concentrations impede the uptake of other nutrients, resulting in a marked decline in root growth. Although not a major focus of this study, we also observed a clear limit to Mn and Zn uptake under high Fe concentrations (data not shown).

Fe Homeostasis in Spinach and Kale

Despite an increase in Fe-EDTA levels beyond what is normally provided during cultivation, Fe uptake did not increase in spinach and kale. We also found that ferric-chelate reductase activity significantly decreased as Fe concentrations increased. Ferric-chelate reductase is a major component responsible for Fe acquisition and metabolism in Strategy I plants (Robinson et al., 1999). Together, these findings demonstrate that the Fe uptake characteristics of spinach and kale are not altered by an increased Fe supply.

The Fe uptake mechanism is activated in roots under Fe-poor conditions (Larbi et al., 2010). In the current study, lowering the Fe-EDTA concentration (Fe-poor conditions) induced the activity of the Fe uptake mechanism. As anticipated, the initial ferric-chelate reductase activity was high, and when the Fe-EDTA concentration was raised, ferric-chelate reductase activity sharply decreased after one day, leading to a simultaneous decrease in leaf Fe content. Indeed, in *Arabidopsis*, ferric-chelate reductase (FRO2) activity was higher under 4 days of cultivation in Fe-poor conditions compared to a 48-hour treatment with 10 μM of Fe-EDTA (Vert et al., 2002). Furthermore, the expression of *FRO2* and *IRT2* (encoding an essential transporter) decreased under Fe-EDTA treatment (Vert et al., 2002). In pea, another Strategy I plant, the expression of the *FRO2* ortholog was strongly induced under Fe-deficient conditions (Waters et al., 2002).

In spinach, we found that leaves in the upper part of the plant had higher Fe contents than lower leaves. This result supports the notion of smooth transfer of Fe within the plant, suggesting that under the environmental conditions used in this study, the shoot Fe content corresponded to the root Fe uptake capability.

For kale, lower (5-200 μM) Fe treatments did not trigger significant differences in shoot Fe contents, whereas higher (> 200 μM) Fe treatments caused an increase. However, shoot growth was stunted under Fe treatments of > 200 μM, such as 400 and 800 μM (data not shown). As ferric-chelate reductase activity was drastically lower in treatments of < 200 μM, we believe that the increase in Fe levels was due to reduced growth, which caused the metal to become concentrated in the plant.

Bioactive Compounds of Spinach and Kale

Typically, Fe is toxic to plants at high concentrations because such conditions trigger ROS production (Schmidt, 2003). Excessive accumulation of other transition metals such as Zn, Ni, Hg, and Mn may also cause oxidative stress and lipid peroxidation. In turn, antioxidant enzyme activity will markedly increase in plants as a defense mechanism (such enzymes include superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and glutathione reductase). Furthermore, excessive metal accumulation also influences antioxidants such as ascorbic acid and glutathione (Cho and Park, 2000; Madhava Rao and Sresty, 2000; Shi et al., 2005; Tewari et al., 2008). However, we observed a decrease in the contents of secondary metabolites (i.e., phenolic compounds) with increasing Fe treatment. This outcome was likely due to the influence of a currently unknown metabolic process that occurs when root Fe uptake is limited. By contrast, root growth in kale decreased with increasing Fe-EDTA concentration, although phenolic levels did not significantly increase. Both results indicate that Fe homeostasis mechanisms in spinach and kale prevent excessive Fe accumulation, making any increase in antioxidant phenolic compound levels unnecessary. The pattern detected in spinach and kale is in contrast to our previous findings for alfalfa, broccoli, and radish seeds (Park et al., 2014). When we soaked these seeds for 5-8 hours in Fe solution (2.5, 5.0, and 10 mM) followed by germination, Fe and total phenolic contents significantly increased in alfalfa sprouts but not in broccoli or radish sprouts (Park et al., 2014). These data suggest that there is considerable cross-species variation in how Fe and secondary metabolite contents respond to environmental Fe levels. Further studies are needed to examine the cause of such differences.

In conclusion, we confirmed that Fe homeostasis is maintained in hydroponically grown spinach and kale under Fe-rich conditions. Specifically, ferric-chelate reductase activity was inhibited in roots under high Fe concentrations. Our results also indicate that there is a species-specific response in plant growth and the production of bioactive compounds, suggesting that it would be useful to perform further comparative studies to examine interspecific differences in Fe homeostasis mechanisms in greater detail in the future.

Acknowledgments: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2014R1A1A1007793). This article was supported by the SMART Research Professor Program of Konkuk University.

Literature Cited

Alscher RG, Donahue JL, Cramer CL (1997) Reactive oxygen species and antioxidants: relationships in green cells. Physiol Plant 100: 224-233

Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic content and other oxidation substrates in plants tissues using folin-ciocalteu reagent. Nat Protoc 2:875-877

Bastide NM, Pierre FHF, Corpet DE (2011) Heme Fe from meat and risk of colorectal cancer: a meta-analysis and a review of the mechanisms involved. Cancer Prev Res 4:177-184

Batty LC, Younger PL (2003) Effects of external Fe concentration upon seedling growth and uptake of Fe and phosphate by the common reed, *Phragmites australis* (Cav.) Trin ex. Steudel. Ann Bot 92:801-806

Bergquist SM, Gertsson UE, Knuthsen P, Olsson ME (2005) Flavonoids in baby spinach (*Spinacia oleracea L.*): changes during plant growth and storage. J Agr Food Chem 53:9459-9464

Bhattacharjee S, Dasgupta P, Paul AR, Ghosal S, Padhi KK, Pandey LP (1998) Mineral element composition of spinach. J Sci Food Agr 77:456-458

Bienfait HF (1989) Prevention of stress in Fe metabolism of plants. Acta Bot. Neerlandica 38:105-129

Bughio N, Takahashi M, Yoshimura E, Nishizawa NK, Mori S (1997) Light-dependent Fe transport into isolated barley chloroplasts. Plant Cell Physiol 38:101-105

Briat JF, Lobréaux S (1997) Fe transport and storage in plants. Trends Plant Sci 2:187-193

Briat JF, Lebrun M (1999) Plant responses to metal toxicity. Comptes Rendus. Acad. Sci. Paris 322:43-54

Campos AM, Lissi EA (1996) Total antioxidant potential of Chilean wines. Nutr Res 16:385-389

Chatterjee C, Gopal R, Dube BK (2006) Impact of Fe stress on biomass, yield, metabolism and quality of potato (*Solanum tuberosum* L.). Sci Hortic 108:1-6

Chavarro JE, Rich-Edwards JW, Rosner BA, Willett WC (2006) Fe intake and risk of ovulatory infertility. Obstet Gynecol 108: 1145-1152

Cho UH, Park JO (2000) Mercury-induced oxidative stress in tomato seedlings. Plant Sci 156:1-9

Grusak MA, Welch RM, Kochlan LV (1990) Physiological characterization of a single-gene mutant of *Pisum sativum* exhibiting excess Fe accumulation. Plant Physiol 93:976-981

Grusak MA (1995) Whole-root Fe(III)-reductase activity throughout the life cycle of Fe-grown *Pisum sativum* L. (Fabaceae): relative to the Fe nutrition of developing seeds. Planta 197:111-117

Havlin JL, Soltanpour PN (1980) A nitric acid plant tissue digest method for use with inductively coupled plasma spectrometry. Commun Soil Sci Plant Anal 11:969-980

Hendry GAF, Brocklebank KJ (1985) Fe-induced oxygen radical metabolism in waterlogged plants. New Phytol 101:199-206

Jin CW, Du ST, Chen WW, Li GX, Zhang YS, Zheng SJ (2009) Elevated carbon dioxide improves plant Fe nutrition through enhancing the Fe-deficiency-induced responses under Fe-limited conditions in tomato. Plant Physiol 150:272-280

Kaluza J, Wolk A, Larsson SC (2013) Heme iron intake and risk of stroke; a prospective study of men. Stroke 44:334-339

Korean Ministry of Health and Welfare (2007) Korea national health and nutrition survey

Larbi A, Morales F, Abadía A, Abadía J (2010) Changes in iron and organic acid concentrations in xylem sap and apoplastic fluid of iron-deficient Beta vulgaris plants in response to iron resupply. J Plant Physiol 167:255-260

Lee MJ, Son JE, Oh MM (2014) Growth and phenolic compounds of *Lactuca sativa* L. grown in a closed-type plant production system with UV-A, -B, or −C lamp. J Sci Food Agr 94:197-204

Madhava Rao KV, Sresty TVS (2000) Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* L. Millspaugh) in

response to Zn and Ni stresses. Plant Sci 157:113-128

Martin MH (1968) Conditions affecting the distribution of *Mercurialis perennis* L. in certain cambridgeshire woodlands. J Ecol 56:777-793

Mills HA, Jones JB (1996) Plant analysis handbook II: a practical sampling, preparation, analysis, and interpretation guide. Micro-Macro Pub. Athens, GA. USA

Montgomery BL, Oh S, Karakkat B (2015) Molecular basis and fitness implications of the interplay between light and the regulation of Fe homeostasis in photosynthetic organisms. Environ Expt Bot 114:48-56

Park SA, Grusak MA, Oh MM (2014) Concentrations of minerals and phenolic compounds in three edible sprout species treated with Fe-chelates during imbibition. Hortic Environ Biotechnol 55:471-478

Robinson NJ, Procter CM, Connolly EL, Guerinot ML (1999) A ferric-chelate reductase for Fe uptake from soils. Nature 397: 694-697

Schmidt W (2003) Fe homeostasis in plants: sensing and signaling pathways. J Plant Nutr 26:2211-2230

Shi Q, Bao Z, Zhu Z, He Y, Qian Q, Yu J (2005) Silicon-mediated alleviation of Mn toxicity in *Cucumis sativus* in relation to activities of superoxide dismutase and ascorbate peroxidase. Phytochemistry 66:1551-1559

Tewari RK, Kumar P, Sharma PN (2008) Morphology and physiology of zinc-stressed mulberry plants. J Plant Nutr Soil Sci 171:286-294

Vert G, Grotz N, Dédaldéchamp F, Gaymard F, Guerinot ML, Briat JF, Curie C (2002) IRT1, an Arabidopsis transporter essential for Fe uptake from the soil and for plant growth. Plant Cell 14:1223-1233

Waters BM, Blevins DG, Eide DJ (2002) Characterization of FRO1, a pea ferric-chelate reductase involved in root Fe acquisition. Plant Physiol 129:85-94

World Health Organization (WHO) (2008) Worldwide prevalence of anemia 1993-2005: WHO global database of anemia. Geneva. Available via http://apps.who.int/iris/bitstream/10665/43894/1/9789241596657_eng.pdf

Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64:555-559