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Effects of nitric oxide and Fe supply on recovery of Fe deficiency induced chlorosis in peanut plants

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Abstract

The effects of nitric oxide (NO) and/or iron (Fe) supplied to Fe deficient plants have been investigated in peanut (*Arachis hypogaea* L.) grown in Hoagland nutrient solution with or without Fe. Two weeks after Fe deprivation, recovery was induced by addition of 250 μM sodium nitroprusside (SNP, a NO donor) and/or 50 μM Fe (Fe-EDTA) to the Fe deprived (-Fe) nutrient solution. Activities of antioxidant enzymes, leaf chlorophyll (Chl), and active Fe content decreased, whereas activities of H^+-ATPase, ferric-chelate reductase (FCR), nitrate reductase, and nitric oxide synthase and NO production increased in Fe deficient plants, consequently an Fe chlorosis symptom appeared obviously. In contrast, these symptoms disappeared gradually after two weeks with NO and/or Fe supply, which caused an increases in leaf Chl and active Fe content, especially following by co-treatment with NO and Fe to values found in Fe sufficient plants. Increased activities of antioxidant enzymes (superoxide dismutase, peroxidase, and catalase) and decreased accumulation of reactive oxygen species (H_2O_2, $O_2^{\bullet-}$) and malondialdehyde enhanced the ability of resistance to oxidative stress. Supplied NO alone had the obvious effect on increased NO production and on activity of H^+-ATPase and FCR, whereas root length and root/shoot ratio were most effectively increased by Fe supplied alone. Co-treatment with NO and Fe did the best effects on recovery peanut chlorosis symptoms by significantly increased Chl and available Fe content and adjusted distribution of Fe and other mineral elements (Ca, Mg, and Zn) in both leaves and roots.

Additional key words: active Fe, antioxidant enzymes, chlorophyll, ferric-chelate reductase, H^+-ATPase, nitrate reductase, nitric oxide synthase, reactive oxygen species.

Introduction

Iron is a very important element in plants for many cellular processes including photosynthesis, respiration, nitrogen fixation (Colangelo and Guerinot 2004), DNA synthesis, and cell differentiation (Graziano and Lamattina 2007). Iron deficiency can block synthesis of chlorophyll (Chl) precursors (pyrrole and porphyrin rings) and accumulation more Fe in the midrib and veins, with a lower Fe concentration in leaf mesophyll (Tomasi *et al*. 2009). Thus, Fe deficiency results in severe chlorosis of plants and finally limits crop production and quality (Briat *et al*. 2014). Despite Fe is very abundant in soil, it exists predominantly as an insoluble ferric-hydroxide complex, which limits the bioavailability of Fe and induces the occurrence of Fe chlorosis symptoms in many plants. In addition, Fe deficiency has been shown to affect expression and activity of certain peroxidase isoenzymes and induce a secondary oxidative stress in dicotyledonous species (Ranieri *et al.* 2001). Further, Fe is an integral constituent or cofactor of many antioxidant enzymes such as catalase (CAT), peroxidase (POD), glutathione reductase, and Fe-superoxide dismutase (Fe-SOD) (Halliwell 2006). Higher plants have

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Abbreviations: Car - carotenoid; CAT - catalase; Chl - chlorophyll; FCR - ferric-chelate reductase; LSD - least significant diffrence; MDA - malondialdehyde; NR - nitrate reductase; NOS - nitric oxide synthase; $O_2^{\bullet-}$ - superoxide anion; PM - plasma membrane; POD - peroxidase; ROS - reactive oxygen species; SNP - sodium nitroprusside; SOD - superoxide dismutase.
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developed two main strategies: the reduction mechanism (strategy I), which is used by dicots and non-graminaceous monocots, and the chelation mechanism (strategy II), which is used by graminaceous monocots, to solubilize and efficiently assimilate Fe when this nutrient is limited (Hell and Stephan 2003).

Iron resupplied to Fe deficient plants restores many plant functions (Larbi *et al.* 2010, Osorio *et al.* 2014), for instance, an increased Chl content and photosynthetic activity in several species including sugar beet (Larbi *et al.* 2004), strawberry (Osorio *et al.* 2014), chickpea (Mahmoudi *et al.* 2007), *etc*. It was also recorded that Fe supply could adjust the composition of mineral elements (Pestana *et al.* 2012) and ferric-chelate reductase (FCR) activity (Larbi *et al.* 2010) during recovery of Fe deficiency.

Nitric oxide is an important signal molecule in plants involved in regulation of seed germination, adventitious root formation, flowering, programmed cell death, stomatal closure (Besson-Bard *et al.* 2008), and responses to salinity (Du *et al.* 2015), heavy metals (Liu *et al.* 2015), hypoxia and high irradiance (Xu *et al.* 2010, Liu *et al.* 2015), *etc*. Under Fe deficiency, NO changes expression of Fe-acquisition genes and morphological and physiological responses to Fe deficiency in cucumber and *Arabidopsis* (García *et al.* 2011). Graziano *et al.* (2002) showed for the first time that treatment with NO prevents the typical interveinal chlorosis in maize grown in Fe deficiency by improved internal Fe availability and an increased Fe pool. Further, NO increases the intracellular availability of Fe by enhancing reduction of Fe^{3+} to Fe^{2+} (Kumar *et al.* 2010) and increasing FCR activity in *Arabidopsis* and cucumber (Chen *et al.* 2010, García *et al.* 2011).

Peanut (*Arachis hypogaea* L.) is a major oil seed crop and ranks a fifth in vegetable oil production worldwide (Shi *et al.* 2014, Attree *et al*. 2015). However, a large proportion of peanut is produced in calcareous soils, so that Fe deficiency chlorosis is a severe and common problem, which greatly limits the yield and quality of peanut (Gao and Shi 2007). Iron uptake in peanut plants belongs to the strategy I, which employs a range of responses to Fe deficiency, including enhanced root branching and subapical root hair development (Chen *et al*. 2010). Further, decrease of pH in root rhizosphere, increase in root plasmalemma FCR activity, release of protons, organic acids, phenolics, *etc.*, are often observed (Morrissey and Guerinot 2009). These processes contribute to chelating Fe^{3+} and converting Fe^{3+} to Fe^{2+} in the plasma membrane (PM) of root epidermal cells. Subsequently, Fe^{2+} is transported into roots *via* specific membrane-bound transporters (IRT1) (Vert *et al*. 2002). After that, Fe is combined with chelators, such as nicotianamine and citrate (Conte and Walker 2011) and transferred from roots to shoots through xylem.

Based on previous studies, we reasoned that supplied NO and/or Fe may have various impacts on recovery from Fe deficiency-induced chlorosis. To understand their effects and mechanisms, we measured plants growth, active Fe and total Fe content, and Fe distribution at the subcellular level in different parts of the plants, as well as the ability of resistance to oxidative stress, and we also measured some key enzymes activities.

Materials and methods

Plants and experiment design: Seeds of peanut (*Arachis hypogaea* L.) were surface sterilized with 5 % (m/v) sodium hypochlorite for 15 min, washed extensively with distilled water, and germinated on moist filter paper in the dark at 25 ºC for 2 d. After germination, uniform seedlings were transferred into aerated half strength Hoagland nutrient solution (Hoagland and Arnon 1950) for 3 d. The nutrition solution was subsequently replaced with full-strength Hoagland solution. When the fifth leaf expanded fully, cotyledons were removed, and the seedlings were transferred into 2 dm^3 black plastic containers (six seedlings per container), and the plants were divided into two batches: with or without 100 μM Fe(III)-EDTA supplied in the nutrition solution. After two weeks, the plants from the Fe(III)-EDTA batch were further grown with Fe(III)-EDTA supplied with different sodium nitropruside (SNP) doses: 0.0 μM SNP (+Fe) and 250 μM SNP (+Fe+SNP). Plants from the other batch without Fe(III)-EDTA were supplied with different SNP and Fe(III)-EDTA doses: 0.0 μM SNP + 0.0 μM Fe(III)-EDTA (-Fe), 250 μM SNP + 0.0 μM Fe(III)-EDTA (-Fe+SNP), 0.0 μM SNP + 50 μM Fe(III)-EDTA (-Fe+Fe_{50}) and 250 μM SNP + 50 μM Fe(III)-EDTA (-Fe+Fe_{50}+SNP). Hoagland solution was adjusted to pH 6.3 and renewed every 3 d. The treatments were arranged in a randomized block design with triplicates. The plants were grown in a growth chamber at a 14-h photoperiod, an irradiance of 300 μmol m^{-2} s^{-1}, day/night temperatures of 25/20 ºC, and a relative humidity of 60 %.

Plant growth parameters and content of Fe and other mineral elements: After 28 d of the treatment, the plants were harvested and separated into roots and shoots, washed with 5 mM $CaCl_2$ first, and repeatedly with deionized water, and then shoot height and root length were measured. For estimation of plant dry matter and content of minerals, the plant samples were oven-dried at 105 ºC for 30 min and at 80 ºC till the materials reached a constant mass. About 0.2 g of the dried material of young leaves, old leaves, stems, and roots were digested in mixed concentrated HNO_3 + $HClO_4$ (3:1, v/v)

supplemented with a few drops of H_2O_2. Total Fe and other mineral element content in the digested solutions were determined using atomic absorption spectrometry (*TAS-990*, *Persee*, Beijing, China) according to Kong *et al*. (2014).

Root activity was expressed as the amount of triphenyl formazan deoxidized by triphenyltetrazolium chloride (Li 2000). Roots were washed thoroughly with distilled water and finally with deionized water and cut into small pieces. Root samples (0.5 g) were placed into test tubes with 5 cm^3 of 0.4 % (m/v) triphenyltetrazolium chloride, and 5 cm^3 of a 1/15 mM phosphate buffer solution (pH 7.0) was added to the tubes and let at 37 °C for 2 h. Then, 2 cm^3 of 1 M H_2SO_4 was added to stop the reaction. Triphenyl formazan was extracted by 95 % (v/v) ethanol for 24 h till the red color faded from the roots.

Photosynthetic pigments: After 20 and 28 d of the treatment, Chl and carotenoid (Car) content of leaves was determined according to the method of Knudson *et al*. (1977). Fresh leaf tissue (0.5 g) was extracted with 2 cm^3 of 95 % (v/v) ethanol in the dark for 24 h, and the extract was analyzed for content of Chl *a,* Chl *b*, and Cars by reading absorbances at 665, 649, and 470 nm, respectively, using a spectrophotometer (*UNICOWF UV-2000*, China)

Isolation of the plasma membrane and measurement of H^+-ATPase activity: A membrane fraction enriched in PM vesicles was prepared as described by Briskin *et al*. (1987) with minor modifications. Excised roots were homogenized with a mortar and pestle in a cold grinding medium (1:2, m/v) containing 25 mM HEPES-Tris (pH 7.2), 250 mM mannitol, 5 mM Na_2-EDTA, 5 mM EGTA, 1 mM DTT, and 1.5 % (m/v) polyvinyl-pyrrolidone. All isolation procedures were carried out at 4 ºC. The homogenate was filtered through four layers of cheesecloth and centrifuged at 560 *g* for 12 min, the supernatant was centrifuged at 10 000 *g* for 15 min, and then the supernatant was separated and again centrifuged at 60 000 *g* for 30 min to yield a crude membrane fraction. The resultant pellet was resuspended in 1 cm^3 of a gradient buffer containing 20 mM HEPES-Tris (pH 7.5), 5 mM Na_2-EDTA, and 0.5 mM EGTA. The supernatant was layered on the top of a step gradient consisting of 1 cm^3 of 45, 33, and 15 % (m/v) sucrose, respectively, and then centrifuged at 70 000 *g* for 2 h. The tonoplast-enriched fraction was collected at the 15/33 % sucrose interface and the PM-enriched fraction was collected at the 33/45 % sucrose interface. Each fraction was centrifuged at 100 000 *g* for 60 min. The resulting pellet was resuspended in a medium containing 20 mM HEPES-Tris (pH 7.5), 3 mM $MgCl_2$, 0.5 mM EGTA, 300 mM sucrose, then quickly frozen in liquid nitrogen, and stored at -70 ºC until used for enzyme assays.

Assays of ATP hydrolysis were performed as described by Briskin *et al*. (1987) in 0.5 cm^3 of a reaction medium containing: 36 mM Tris-Mes (pH 6.5), 30 mM Na_2-ATP, 3 mM $MgSO_4$, 1 mM NaN_3, 50 mM KNO_3, 1 mM Na_2MoO_4, 0.02 % (m/v) *Triton A-100*, in the presence or absence of 2.5 mM Na_3VO_4. The reaction was triggered by adding 0.05 cm^3 of the PM vesicles. After 30 min incubation at 37 ºC, the reaction was quenched by addition of 0.05 cm^3 of 55 % (m/v) trichloroacetic acid. Activity of H^+-ATPase was determined by measuring release of Pi (Ohinishi *et al*. 1975).

Soluble protein content was measured by the Coomassie brilliant blue G-250 method (Bradford 1976). Coomassie Brilliant Blue G-250 (100 mg) was dissolved in 50 cm^3 of 95 % (v/v) ethanol, and 100 cm^3 of 85 % (m/m) phosphoric acid was added to prepare Bradford reagent. The sample (0.1 cm^3) and 3 cm^3 of the reagent were mixed and after 2 min, absorbance was read at 595 nm.

Ferric-chelate reductase activity: After 20 and 28 d of the treatment, plant roots were immersed in a saturated $CaSO_4$ solution for 5 min, washed with deionized water, and then placed in a beaker with a nutrient solution containing 0.1 mM Fe(III)-EDTA and 0.4 mM 2,2'-bipyridyl, pH 5.0. The environmental conditions during the measurement were the same as for plant growth. After 2 h, FCR capacity was determined by measuring the amount of an Fe(II)-bipyridyl complex formed (Gao and Shi 2007).

Active Fe content and Fe subcellular distribution: After 20 and 28 d of the treatment, young and old leaves, stems, and roots were cut into pieces and 2.0 g of samples were extracted with 20 cm^3 of 1 M HCl (1:10, m/v, tissue:extractant) (Takker and Kaur 1984), shaken for 5 h, filtered, and subsequently active Fe content in the filtrate was measured using an atomic absorption spectro-photometer (Gao and Shi 2007).

For Fe subcellular distribution, roots were separated and immersed in 20 mM Na_2-EDTA for 15 min and then rinsed three times with deionized water to remove any Fe attached to the external surface of the roots. Fresh samples (25 g) of young leaves, old leaves, stems, and roots were homogenized in 50 cm^3 of a chilled extraction buffer containing 50 mM HEPES (pH 7.5), 500 mM sucrose, 1 mM DTT, 5 mM ascorbate, and 1 % (m/v) polyvinylpyrrolidone. The homogenate was centrifuged at 500 *g* for 5 min to isolate the cell wall fraction. The supernatant from this centrifugation step was then centrifuged at 20 000 *g* for 45 min to sediment cell organelles, and the resultant supernatant solution was referred to as the soluble fraction. All steps were performed at 4 ºC. The fractions of the samples were digested in a mixture of concentrated HNO_3 and $HClO_4$ (4:1, v/v) at 120 ºC for at least 3 h. Amount of Fe was then determined using an atomic absorption spectrometer (Su *et al*. 2014).

Antioxidant enzyme activities and malodialdehyde content: After 28 d of the treatment, roots and the second fully expanded leaves of the plants were sampled for enzymatic analysis. The samples were homogenized in a 50 mM phosphate buffer (pH 7.8) by grinding in a mortar and pestle under chilled conditions with liquid nitrogen. The homogenate was filtered through four layers of muslin cloth and centrifuged at 12 000 *g* and 4 ºC for 10 min. All spectrophotometric analyses were conducted on a spectrophotometer *UNICOWF UV-2000*. Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit photochemical reduction of nitroblue tetrazolium following the method of Stewart and Bewley (1980). One unite of SOD activity was defined as the amount of the enzyme required to cause a 50 % inhibition of reduction of nitroblue tetrazolium monitored at 560 nm. Catalase (CAT) activity was measured as decline in absorbance at 240 nm due to the decrease of absorbance of H_2O_2 according to the method of Patra *et al*. (1978). The enzyme activity was calculated using the coefficient of absorbance of 39.4 $mM^{-1}cm^{-1}$. Peroxidase (POD) activity was measured by increase in absorbance at 470 nm due to guaiacol oxidation (Nickel and Cunningham 1969). One unit of POD was defined as an absorbance change of 0.01 per minute. Lipid peroxidation was measured in terms of malondialdehyde (MDA) content by the thiobarbituric acid reaction method (Heath and Packer 1968).

Hydrogen peroxide content and superoxide anion generation rate: For determination of H_2O_2 content, 0.2 g of fresh leaves or roots were extracted with 3 cm^3 of 0.1 % (m/v) trichloroacetic acid in an ice bath and then centrifuged at 12 000 *g* for 15 min (Velikova *et al.* 2000). An aliquot (0.5 cm^3) of the supernatant was added to 0.5 cm^3 of a 10 mM phosphate buffer (pH 7.0) and 1 cm^3 of 1 M KI. The absorbance of the mixture was read at 390 nm. Content of H_2O_2 was determined using the coefficient of absorbance of 0.28 μM^{-1} cm^{-1}.

For measurement of $O_2^{\bullet -}$ generation rate, 0.3 g of fresh leaves or roots were homogenized in 3 cm^3 of an ice cold 50 mM phosphate buffer (pH 7.0) and the homogenate was centrifuged at 10 000 *g* for 10 min. Then, 0.5 cm^3 of the supernatant was added to 0.5 cm^3 of a 50 mM phosphate buffer (pH 7.8) and 0.1 cm^3 of 10 mM hydroxylamine hydrochloride. The mixture was incubated at 25 ºC for 35 min. Then, 0.5 cm^3 of the mixture was added to 0.5 cm^3 of 17 mM sulfanilamide and 0.5 cm^3 of 7.8 mM α-naphthylamine. After 20 min of reaction, 2 cm^3 of ether was added into the solution and mixed well. The solution was centrifuged at 1500 *g* and 4 ºC for 5 min. The absorbance of the pink supernatant was measured at 530 nm. Absorbance values were calibrated to a standard curve generated with known concentrations of HNO_2 (Shi and Zhu 2008).

Nitrate reductase and nitric oxide synthase activities and NO content: NR activity was measured following the method adopted by Jaworski (1971). Fresh leaf and root samples were cut into small pieces (approximately 1 mm) and transferred to plastic vials containing a phosphate buffer (pH 7.5). Then, solutions of 0.1 mM potassium nitrate and 1 % (v/v) isopropanol were added. The reaction mixture was incubated at 30 °C for 2 h followed by addition of N-1-naphthylethylenediamine dihydrochloride and sulfanilamide. Absorbance was read at 540 nm and was compared with that of a calibration curve.

Activity of nitric oxide synthase (NOS) was measured using a commercial kit (Xiong *et al.* 2001). An aliquot of the supernatant was incubated with L-arginine, the substrate for NOS, an accelerator, and a developer at 37 °C for 15 min. Afterwards, a clearer and a stop buffer were added to the reaction volume, and then NO production was determined at 530 nm. A commercial assay kit of NOS was obtained from the Nanjing Jiancheng Biological Medical Engineering Institute (Nanjing, China).

Nitric oxide was assayed using the spectrofluorimetric method (Airaki *et al.* 2012). A 2 μM 4,5-diamino-fluorescein diacetate was added to the crude extracts of leaves and roots. The reaction mixtures were then incubated at 37 ºC in the dark for 2 h, and fluorescence was measured in a *QuantaMaster*^{TM}*QM-4* (*Photon Technology International*, USA) fluorescent spectro-photometer at excitation and emission wavelengths of 485 and 515 nm, respectively.

Statistical analysis: Analysis of variance (*ANOVA*) was performed using the *SAS* software (*SAS Institute*, Cary, NC, USA). Differences between treatments were separated by the least significant difference (LSD) test at a 0.05 probability level.

Results

Compared to the Fe sufficient plants, shoot height, root length, dry mass, and root activity significantly decreased in the Fe deficient plants (Table 1). As compared with the Fe deficient plants, NO and/or Fe supply increased root length and dry mass. Especially, Fe supply significantly increased root length and root activity by 7.79 and 28.5 %, whereas dry mass increased by 15.9 % in the -Fe+Fe_{50}+SNP treatment. In contrast, root/shoot ratio decreased in the -Fe+SNP and -Fe+Fe_{50}+SNP treatments, but significantly when Fe was supplied.

Nitric oxide supply had no obvious effect on Chl content at 20 and 28 d in the Fe sufficient plants. Typical

Table 1. Effects of NO and/or Fe supply on shoot height, root length [cm plant^{-1}], dry mass [g plant^{-1}], root/shoot ratio, and root activity [μg(triphenyl formazan) g^{-1}(f.m.) min^{-1}] in 28 d treated peanut plants. Means ± SDs, n = 3. Different letters within the same column indicate significant differences at $P < 0.05$ according to the LSD test.

Treatment	Shoot height	Root length	Dry mass	Root/shoot ratio	Root activity
+Fe	15.23 ± 1.23a	9.30 ± 0.46a	0.79 ± 0.02a	0.42 ± 0.002c	1.52 ± 0.04a
+Fe+SNP	14.77 ± 0.50a	9.40 ± 0.10a	0.75 ± 0.01b	0.42 ± 0.002c	1.26 ± 0.02b
-Fe	11.80 ± 0.61b	8.47 ± 0.25c	0.63 ± 0.01d	0.44 ± 0.005b	1.09 ± 0.04d
-Fe+SNP	12.37 ± 0.15b	8.73 ± 0.15bc	0.68 ± 0.01c	0.42 ± 0.001c	1.01 ± 0.04c
-Fe+Fe_{50}	12.50 ± 0.60b	9.13 ± 0.25ab	0.70 ± 0.01c	0.45 ± 0.003a	1.29 ± 0.01b
-Fe+Fe_{50}+SNP	12.87 ± 0.21b	8.97 ± 0.12ab	0.73 ± 0.02b	0.42 ± 0.001c	1.07 ± 0.03c

Table 2. Effects of NO and/or Fe supply on chlorophyll (Chl) *a,* Chl *b*, and carotenoids (Cars) content [mg g^{-1} (f.m.)] and Chl *a/b* ratio in 20 and 28 d treated peanut plants. Means ± SDs, n = 3. Different letters within the same column indicate significant differences at $P < 0.05$ according to the LSD test.

Treatment	20 d				28 d			
	Chl *a*	Chl *b*	Cars	Chl *a*/*b*	Chl *a*	Chl *b*	Cars	Chl *a*/*b*
+Fe	1.81±0.06a	0.65±0.02a	0.34±0.02c	2.76±0.02b	2.06±0.03a	0.60±0.01a	0.48±0.01a	3.42±0.12b
+Fe+SNP	1.80±0.04a	0.65±0.01a	0.34±0.01c	2.76±0.11b	2.11±0.05a	0.61±0.01a	0.46±0.01a	3.45±0.09b
-Fe	1.47±0.05c	0.42±0.01c	0.34±0.01c	3.46±0.13a	1.45±0.07d	0.37±0.01d	0.33±0.01d	3.96±0.26a
-Fe+SNP	1.47±0.05c	0.44±0.02c	0.41±0.02ab	3.33±0.28a	1.68±0.03c	0.46±0.01c	0.39±0.01c	3.68±0.04b
-Fe+Fe_{50}	1.66±0.02b	0.49±0.02b	0.39±0.01b	3.42±0.11a	1.92±0.09b	0.56±0.01b	0.44±0.01b	3.42±0.22b
-Fe+Fe_{50}+SNP	1.70±0.09b	0.50±0.01b	0.42±0.01a	3.37±0.13a	2.11±0.03a	0.61±0.01a	0.48±0.01a	3.43±0.03b

symptoms of Fe deficiency appeared progressively in young leaves in the Fe deficient plants: a significantly decreased content of Chl *a* and Chl *b*, but increased Chl *a/b* ratio as compared to the Fe sufficient plants. Addition of Fe significantly increased Chl *a*, Chl *b*, and Car content, especially in the -Fe+Fe_{50}+SNP, but Chl *a/b* decreased. When NO was supplied alone, an increased total Chl content was observed at 28 d, but it was still lower than in the Fe supplied plants (Table 2).

The iron deficient plants had a considerably higher H^+-ATPase activity in leaves and roots than the Fe sufficient plants (Fig. 1*A*). Compared to the -Fe plants, the -Fe+SNP and -Fe+Fe_{50} treatments significantly stimulated activity of H^+-ATPase in leaves, but in roots, it was accomplished only by NO addition. Nitric oxide also increased H^+-ATPase activity in leaves of the +Fe plants.

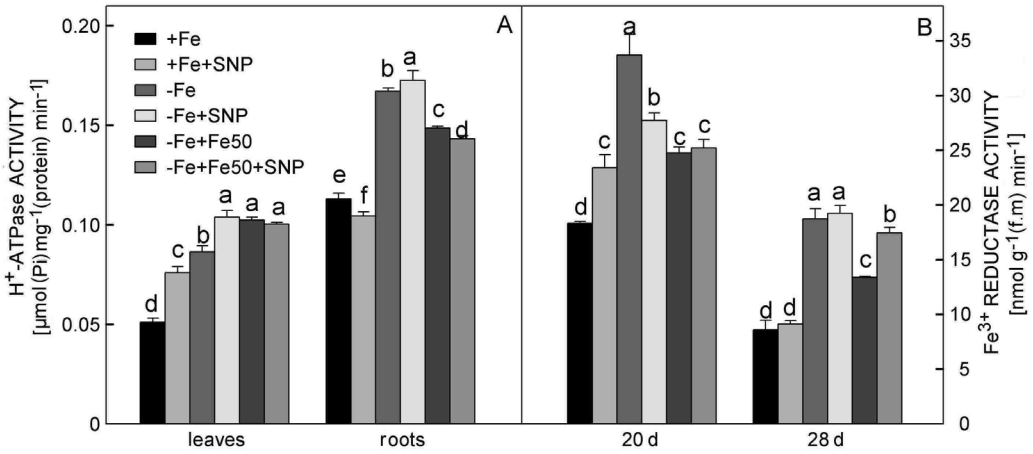

Fig. 1. Effects of NO and/or Fe supply on H^+-ATPase activity in leaves and roots (*A*) of 20 d, and root ferric-chelate reductase activity (*B*) of 20 and 28 d treated peanut plants. Means ± SDs, n = 3. Different letters indicate significant differences at $P < 0.05$ according to the LSD test.

Table 3. Effects of NO and/or Fe supply on active Fe content [mg kg^{-1}(f.m.)] in young leaves, old leaves, stems, and roots of 20 and 28 d treated peanut plants. Means ± SDs, $n = 3$. Different letters within the same column indicate significant differences at $P < 0.05$ according to the LSD test.

Treatment	20 d				28 d			
	young leaves	old leaves	stems	roots	young leaves	old leaves	stems	roots
+Fe	56.09±1.12b	71.07±1.60a	9.08±0.42c	76.73±1.43a	67.58±0.51a	72.05±1.21ab	20.14±0.25a	105.22±1.43b
+Fe+SNP	63.03±2.45a	68.74±0.28a	13.86±0.47a	72.29±1.07a	66.94±138a	73.01±0.76a	20.19±1.29a	103.61±0.50b
-Fe	31.51±2.27e	45.48±2.34d	7.37±0.18e	38.30±1.48d	28.47±0.74d	36.47±1.27e	6.74±0.07c	37.64±0.72d
-Fe+SNP	33.95±1.42e	51.36±1.76c	8.37±0.25d	50.13±0.86c	42.91±0.37c	61.79±0.79d	14.35±1.21b	61.23±1.53c
-Fe+Fe_{50}	38.43±1.25d	49.90±0.57c	10.27±0.18b	50.03±5.93c	51.05±0.74b	64.74±1.06c	13.00±0.89b	103.56±1.83b
-Fe+Fe_{50}+SNP	51.10±1.22c	55.39±0.67b	10.26±0.33b	60.71±0.87b	67.16±0.83a	70.25±1.03b	14.52±0.93b	112.97±0.81a

A markedly increased FCR activities in roots by 52.6 % at 20 d and 117.8 % at 28 d were observed in the -Fe plants compared with the +Fe plants. Supplied NO alone decreased FCR activity at 20 d compared with the -Fe plants; the opposite result appeared in the +Fe plants. Activity of FCR revealed an opposite tendency in the Fe addition treatments, but it was still higher than in the Fe sufficient plants. Furthermore, activity of FCR at 28 d was lower than at 20 d in this experiment (Fig. 1*B*).

Iron deficiency significantly decreased content of active Fe in young leaves, old leaves, stems, and roots in the two sampling dates (20 and 28 d, Table 3). Supplied NO and/or Fe significantly increased active Fe content in the aforesaid parts of the plants, and the Fe supplied plants contained more active Fe than the NO supplied plants. Obviously, the NO and Fe supplied plants had active Fe content similar to the Fe sufficient plants. What is more, content of active Fe also showed a slight increase in young (20 d) and old (28 d) leaves with NO supplied to the +Fe plants.

In the +Fe plants, there were no obvious differences in the total Fe content between NO supplied or not supplied. However, in the -Fe plants, addition of NO increased the total Fe content in young and old leaves but decreased in stems and roots, whereas the total Fe content increased in all parts under the -Fe+Fe_{50} or -Fe+Fe_{50}+SNP treatment compared to the -Fe (Table 4).

Iron deficiency induced changes in mineral element compositions of peanut young leaves, old leaves, stems, and roots (Table 4). The iron deficient plants contained a higher content of Zn in the aforesaid parts and a lower content of Ca and Mg than the +Fe plants. Supplied NO significantly increased content of Ca in shoots and Mg in roots, meanwhile decreased Zn content in both shoots and roots. Supplied Fe and Fe combined with NO significantly increased content of Ca in leaves and roots and decreased content of Zn in the aforesaid parts. However, only the co-treatment with NO and Fe slightly

Table 4. Effects of NO and/or Fe supply on the total content of Fe [mg kg^{-1}(d.m)], Ca [g kg^{-1}(d.m)], Mg [g kg^{-1}(d.m)], and Zn [mg kg^{-1} (d.m)] in young leaves, old leaves, stems, and roots after 28 d treatment of peanut seedlings. Means ± SDs, $n = 3$. Different letters within the same row indicate significant differences at $P < 0.05$ according to the LSD test.

Mineral		+Fe	+Fe+SNP	-Fe	-Fe + SNP	-Fe+Fe_{50}	-Fe+Fe_{50}+SNP
Fe	young leaves	497.13±5.52a	502.94±1.75a	185.84±2.94e	268.92±9.57d	354.97±9.89c	418.49±2.55b
	old leaves	275.69±5.57a	268.38±5.20a	153.79±4.14d	186.73±1.89c	230.01±2.65b	271.21±8.66a
	stems	244.35±5.63c	243.95±4.65c	243.14±11.7c	231.43±11.0c	289.80±3.02a	272.23±13.0b
	roots	1737.64±23.6a	1750.10±29.9a	559.74±15.7d	450.23±8.62e	1156.22±45.3c	1620.31±21.3b
Ca	young leaves	6.13±0.08b	7.71±0.16a	3.60±0.13f	5.51±0.06c	4.29±0.08d	4.00±0.08e
	old leaves	16.39±0.11b	16.84±0.21b	11.58±0.43d	17.48±0.15a	14.28±0.33c	14.44±0.20c
	stems	4.07±0.03c	3.84±0.08d	3.87±0.10d	4.81±0.09b	3.98±0.13cd	5.25±0.16a
	roots	2.60±0.05a	2.14±0.06b	1.71±0.11c	1.75±0.07c	2.11±0.03b	2.12±0.03b
Mg	young leaves	0.86±0.01b	0.95±0.01a	0.81±0.01c	0.81±0.01c	0.81±0.01c	0.85±0.01b
	old leaves	0.93±0.02a	0.93±0.01a	0.90±0.05a	0.92±0.01a	0.92±0.03a	0.92±0.01a
	stems	0.80±0.02a	0.78±0.02a	0.69±0.01c	0.78±0.01a	0.68±0.01c	0.74±0.01b
	roots	1.26±0.02b	1.23±0.01b	1.07±0.03d	1.30±0.01a	1.07±0.02d	1.13±0.03c
Zn	young leaves	17.49±0.38d	18.89±0.60c	26.98±1.27a	21.43±0.67b	16.63±0.91d	17.93±0.22cd
	old leaves	17.19±1.05d	19.73±1.14c	36.31±1.12a	27.35±1.35b	19.37±1.42c	19.36±0.70c
	stems	8.69±0.36b	6.86±0.51d	12.78±0.43a	6.78±0.18d	5.55±0.03e	7.60±0.0.07c
	roots	18.20±0.82c	17.45±1.17c	33.03±1.77a	23.64±1.54b	19.53±1.25c	19.06±0.87c

increased content of Mg. Besides, in the +Fe plants, content of Ca, Mg, and Zn increased in young leaves and decreased in roots after NO addition.

The subcellular distribution of Fe in young leaves, old leaves, stems, and roots (Table 5) shows that addition of NO increased Fe content in cell organelles, but decreased it in cell walls or soluble fractions in the four parts of the +Fe plants. In the Fe deficient plants, NO supplied alone increased Fe content in all parts of shoots, but decreased it in roots. Content of Fe in subcellular fractions had a strongly positive correlation with Fe supplied to the plants. Especially, Fe with NO significantly increased content of Fe in cell organelles by 47.7 % in young leaves, 8.30 % in old leaves, 12.4 % in stems, and 30.6 % in roots as compared to the -Fe+Fe_{50} alone, and even reached Fe content of the +Fe plants. Furthermore, the percentage of Fe in cell organelles + in soluble fractions was higher than in cell walls in stems and roots, but not in old leaves in each treatment of the experiment.

Table 5. Effects of NO and/or Fe supply on subcellular distribution of Fe [mg kg^{-1}(f.m)] in young leaves, old leaves, stems, and roots after 28 d treatment of peanut plants. Means ± SDs, n = 3. Different letters within the same row indicate significant differences at $P < 0.05$ according to the LSD test.

Organ	Fraction	+Fe	+Fe+SNP	-Fe	-Fe + SNP	-Fe+Fe_{50}	-Fe+Fe_{50}+SNP
Young leaves	cell wall	59.07±0.21ab	60.46±0.66a	30.05±0.30e	37.92±1.23d	55.48±1.05c	57.42±2.24bc
	cell organelle	33.20±1.44c	39.05±0.77a	18.24±1.19e	20.07±0.90e	24.22±1.46d	35.78±0.27b
	soluble fraction	23.01±0.50a	21.85±1.69a	17.09±0.98c	19.22±0.43b	21.36±1.27a	21.08±1.18ab
Old leaves	cell wall	69.33±0.84a	63.88±1.62b	44.67±1.41e	48.25±1.99d	64.17±0.77b	60.11±1.47c
	cell organelle	26.04±0.83b	28.60±0.99a	17.08±0.18e	21.64±0.83d	23.14±0.80c	25.06±0.63b
	soluble fraction	27.79±0.85b	27.97±0.51b	20.69±0.43d	24.22±1.46c	26.84±0.36b	31.91±1.05a
Stems	cell wall	31.50±0.67b	37.36±1.78a	20.69±0.33d	24.53±0.77c	22.05±0.49d	32.80±0.92b
	cell organelle	18.12±0.23b	19.75±1.05a	10.86±0.22e	12.86±0.98d	15.10±0.44c	16.97±0.82b
	soluble fraction	17.91±0.88ab	19.01±0.74a	12.17±0.42d	17.49±0.87ab	15.29±1.38c	17.08±0.78b
Roots	cell wall	87.52±1.28a	88.79±0.26a	49.43±1.16c	42.67±1.48d	73.89±0.15b	87.16±0.95a
	cell organelle	26.40±0.05b	26.59±0.47b	20.91±0.30d	19.83±0.71d	24.72±1.04c	32.29±1.71a
	soluble fraction	68.40±0.85a	64.81±1.02b	31.88±1.13c	29.57±0.58d	54.50±1.05d	62.81±0.90c

In the Fe deficient plants, activity of SOD rose about 22.1 and 26.2 % in leaves and roots as compared to the +Fe plants (Fig. 2*A*), whereas an opposite tendency was observed in activities of POD and CAT (Fig. 2*B,C*). As compared to the -Fe plants, NO supplied alone significantly increased SOD activity by 6.28 % in leaves, whereas activities of POD and CAT significantly increased by 8.54 and 32.3 % in roots. In the -Fe+Fe_{50} and -Fe+Fe_{50}+SNP treatments, activity of POD and CAT significantly increased in leaves, whereas in roots only CAT activity significantly increased as compared to the -Fe plants. Activity of SOD in the -Fe+SNP plants was higher than in the -Fe+Fe_{50} in contrast to POD and CAT activities. In the +Fe plants, NO supplied also increased activity of SOD and POD in both leaves and roots.

Iron deficiency significantly increased accumulation of MDA in roots, and $O_2^{\bullet-}$generation rate and H_2O_2 content in both leaves and roots (Fig. 2*D,E,F*). Supplied NO and/or Fe decreased content of MDA and H_2O_2 as well as $O_2^{\bullet-}$generation rate in both leaves and roots. In leaves, Fe and NO in combination was most effective in reducing $O_2^{\bullet-}$generation rate and H_2O_2 content, whereas in roots, NO supplied alone had the best effect on MDA and H_2O_2 content, but Fe supplied alone had the best effect on $O_2^{\bullet-}$generation rate.

Compared to the Fe sufficient plants, activities of NOS and NR significantly increased by 44.8 and 31.4 % in leaves, 114 and 107 % in roots in the Fe deficient plants (Fig. 3*A,B*). Supplied NO and/or Fe decreased NOS and NR activities. Especially, the co-treatment with Fe and NO markedly reduced activities of NOS and NR by 31.1 and 17.7 % in leaves and 39.5 and 20.6 % in roots, respectively, as compared to the Fe deficient plants, but still remained higher than in the Fe sufficient plants. In the Fe sufficient plants, activity of NOS was obviously enhanced in the presence of NO, whereas it had no obvious influence on NR activity.

Iron deficiency caused a significant increase in NO content in both leaves and roots (Fig. 3*C*). Nitric oxide supplied alone markedly increased production of NO by 7.41 % in leaves and 20.4 % in roots as compared with the Fe deficient plants. Similarly, production of NO increased in the presence of NO in the Fe sufficient plants. Compared with the Fe deficient plants, the inhibition of NO generation appeared in the Fe supplied plants, whereas the co-treatment with NO and Fe had no significant effect on NO generation.

Discussion

Iron deficiency often causes imbalance in mineral composition in different parts of plants and decreases leaf Chl content (Pereira *et al*. 2014). We observed a large chlorosis in the Fe deficient plants, which was proved by a large decrease in Chl *a* and *b* content (Table 2). Iron deficiency does not decrease all photosynthetic pigments to the same extent, Cars being less affected than Chl, and Chl *b* is more affected than Chl *a* (Larbi *et al*. 2004). Similarly, ratio of Chl *a/b* increased in the Fe deficient plants (Table 2). The decrease of Chl could increase the susceptibility of photosystem II towards photoinhibition (Pätsìkkä *et al*. 2002) and reduction in the number of photosynthetic units per area (Larbi *et al*. 2006), which consequently decreased sugar synthesis and subsequently shoot height, root length, and dry mass (Table 1). Supplied NO or Fe resulted in leaf re-greening and

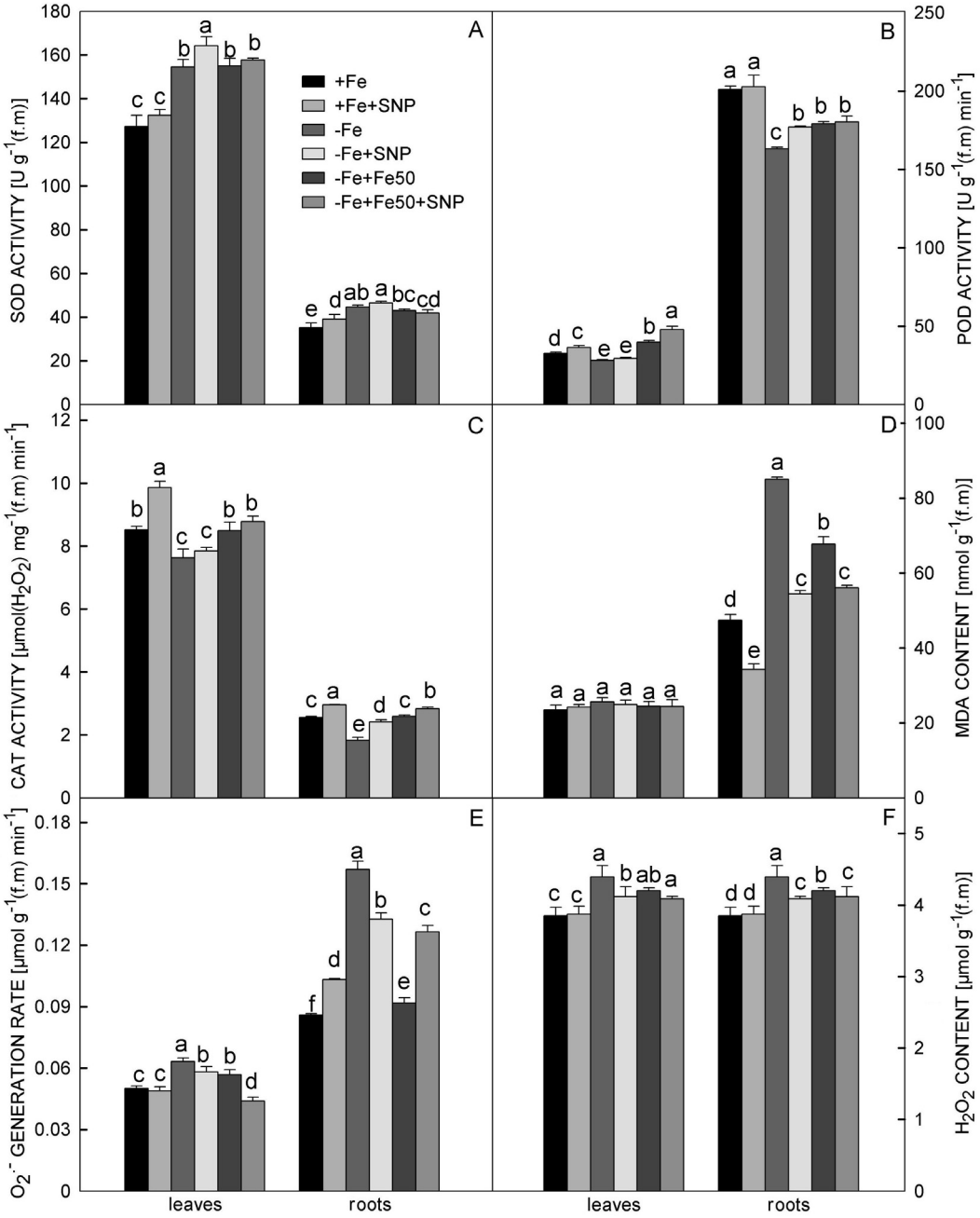

Fig. 2. Effects of NO and/or Fe supply on activities of superoxide dismutase (SOD, *A*), peroxidase (POD, *B*), and catalase (CAT, *C*), on accumulation of malondialdehyde (MDA, *D*), generation rate of $O_2^{\bullet -}$ (*E*), and content of H_2O_2 (*F*) in leaves and roots of 28-d-old in peanut seedlings. Means ± SDs, $n = 3$. Different letters indicate significant differences at $P < 0.05$ according to LSD test.

in increasing photosynthetic rate, especially in the co-treatment with Fe and NO. This is in accordance with Fe supplied to Fe deficient chickpea and strawberry (Mahmoudi *et al*. 2007, Osorio *et al*. 2014), and application of NO to maize, Chinese cabbage, and peanut (Sun *et al*. 2007, Ding *et al*. 2008, Kong *et al*. 2014). Supplied Fe increased content of available Fe in leaves (Table 3) in agreement with previous studies (Mahmoudi *et al*. 2007). Besides, NO could protect Chl from degradation in the Fe deficient plants by increasing Mg content (Table 4) and active Fe content in leaves. Similarly, Kumar *et al.* (2010) have evidenced that NO could increase intracellular availability of Fe. Therefore, the combination of Fe and NO did the best effect on recovering chlorosis of peanut and improving biomass productivity in the Fe deficient plants.

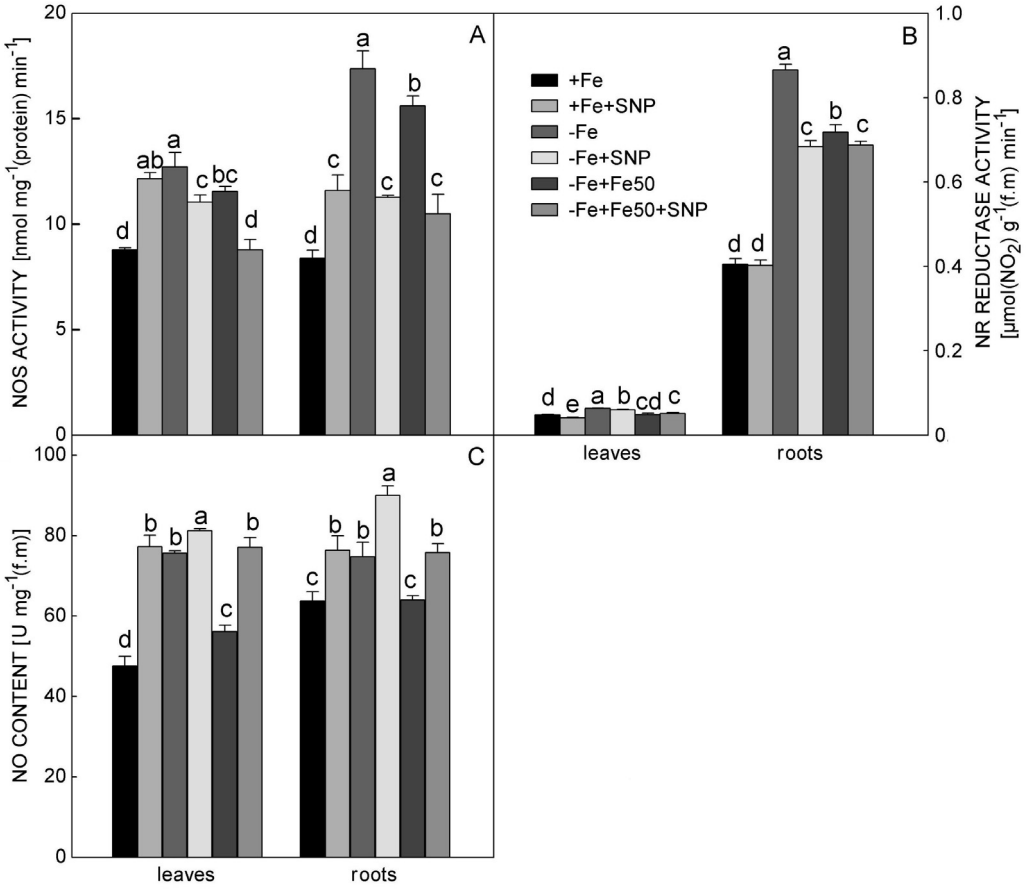

Fig. 3. Effects of NO and/or Fe supply on activities of NOS (*A*) and NR (*B*), and NO content (*C*) in leaves and roots of 28 d treated peanut seedlings. Fluorescence (in *C*) is expressed as arbitrary units (U) per mg of fresh mass (f.m.). Means ± SDs, $n = 3$. Different letters indicate significant differences at $P < 0.05$ according to the LSD test.

Plant roots are active absorption organs, and root growth and activity directly affect nutrition and yield of aboveground parts. After Fe starvation, plants have evolved a series of morphological and physiological adaptations to increase Fe uptake by increasing its solubility and availability in rhizosphere (Hindt and Guerinot 2012). The common response of higher plants to a low Fe-stress is inhibition of root elongation, increased apical root zone, and abundant root hair formation (Schmidt 2003). In peanut, iron deficiency decreased root length and root activity but increased root/shoot ratio, and Fe and/or NO supply alleviated these effects (Table 1). This is in accordance with a previous study, in which NO regulates root tip elongation, adventitious root formation, and root hair development by mediating auxin-regulated processes (Fernández-Marcos *et al*. 2011). The supplied Fe enhanced content of Fe, which may stimulate root activity and increase the absorptive surface area of roots. Similar effects have been reported in sugar beet and chickpea (López-Millán *et al*. 2001, Mahmoudi *et al*. 2007). According to physiological changes in strategy I plants, Fe mobilization is achieved by a combined action of proton-extruding H^+-ATPase and FCR. Since Fe^{3+} should be reduced to Fe^{2+} by PM-bound FCR prior to uptake by roots in strategy I plants (Kobayashi and Nishizawa 2012), and the reduction has been elucidated as a rate-limiting step in Fe uptake (Ishimaru *et al*. 2007), so activity of FCR is important for an efficient uptake, activation, and distribution of Fe in plants. Activities of H^+-ATPase and FCR increased in the

Fe deficient peanut plants (Fig. 1); a similar tendency was occurred in strawberry and cucumber (Pestana *et al*. 2012, Pii *et al*. 2015). Nevertheless, Larbi *et al*. (2010) showed contrary results; root FCR activity significantly decreases in Fe deficient *Beta vulgaris* plants and the differences could be ascribed to treatment duration and species. Activities of H^+-ATPase and FCR were most effectively stimulated in the NO supplied plants but decreased in roots of the Fe supplied plants, and remained still higher than in the +Fe plants. The Fe supplied plants can take up more Fe from rhizosphere and translocate it to shoots, whereas the NO supplied plants could take Fe only from apoplast and other Fe pools in leaves and roots. Further, NO seems to be involved in auxin-dependent activation of FCR in *Arabidopsis* (Bacaicoa *et al*. 2011), and NO may act as a signal activating FCR through a Fe-induced transcription factor (FIT)-mediated transcriptional regulation of ferric oxidoreductase (*FRO2*) (Chen *et al*. 2010). Therefore, activities of H^+-ATPase and FCR were higher in the NO supplied plants than in the Fe supplied plants (Fig. 1). A higher activity of FCR would make more Fe^{3+} reduced to Fe^{2+} in the PM, and then Fe^{2+} was transferred to cells by an iron-regulated transporter belonging to the ZIP family of metal transporters (Vert *et al*. 2002). Besides, plant responses are mainly regulated by nutrient status in shoots in a nutrient deficiency stress (García-Mina *et al*. 2013). Activity of FCR at 28 d after the treatments was lower than at 20 d. A similar decrease in FCR activity has been reported in strawberry leaves and roots (Pestana *et al*. 2012). A similar result was also pointed out by Graziano and Lamattina (2007) in tomato, and by García *et al*. (2011) in *Arabidopsis* and cucumber, where NO applied enhanced FCR activity under Fe-deficient conditions.

Content of the total Fe in young and old leaves severely decreased under Fe deficiency and increased rapidly after Fe re-supply, especially when combined with NO (Table 4). A rapid Fe content increase upon Fe supply has been reported in sugar beet (López-Millán *et al*. 2001, Larbi *et al*. 2010). Nitric oxide has a high affinity towards the Fe-containing active sites of many proteins and thus participates in regulation of Fe transport in plants (Ramirez *et al*. 2010). In this study, NO supplied alone also increased the total Fe content in peanut leaves (Table 4); similar results have been found in *Solanum nigrum* and *S. lycopersicum* (Graziano and Lamattina 2007, Xu *et al*. 2009), but NO does not increase the total Fe content in *Arabidopsis* (Connolly *et al*. 2003). As concern roots, the total Fe content was higher in the Fe supplied plants than in the NO supplied plants (Table 4). Since NO stimulates Fe translocation from roots to shoots, but roots were not able to absorb Fe from the nutrient solution in the NO alone supplied plants, root Fe content was low. However, in the Fe supplied plants, roots could take up a small amount of Fe from the nutrient solution. Thus, Fe combined with NO increased the total Fe content in different parts of the plants but decreased it in roots. Active Fe is considered a better nutritional Fe indicator than the total Fe (Hakan and Vahap 2007). Supplied NO and/or Fe increased active Fe content in leaves, stems, and roots (Table 3). This might be modulated by expression of different genes to affect of Fe uptake and release by protein ferritin (Briat *et al*. 2010, Chen *et al*. 2010). Jasid *et al*. (2008) also found a stress protective effect of the incubation of sorghum seeds in the presence of NO donors by an increase of Fe availability.

In plants, NO reverts the symptoms of Fe deficiency probably by increasing the availability of internal Fe or facilitating delivery of Fe through formation of mono- and di-nitrosyl Fe complexes (Graziano *et al*. 2002, Ramirez *et al*. 2011). Supplied NO increased Fe content in cell organelles in contrast to cell walls or soluble fractions in the Fe sufficient plants, and increased the percentage of Fe in cell organelles and soluble fractions in both leaves and stems but decreased it in roots of the Fe deficient plants (Table 5). This might be attributed to an enhanced ability of reduction of Fe^{3+} to Fe^{2+} by increased activities of H^+-ATPase and FCR (Fig. 1) that make Fe to be easily absorbed from root apoplast and transported in xylem in the Fe deficient plants. On the other hand, NO can bind strongly to Fe to form an Fe-nitrosyl complex (Ueno and Yoshimura 2000), which more easily diffuses through the membrane or can be transported by a PM carrier (Simontacchi *et al*. 2012) so that more Fe could be transferred from the cell wall to cell organelles and soluble fractions. Previous studies have evidenced the presence of Fe-nitrosyl complexes as one of the important components of the labile iron pool (LIP), which participate in Fe cellular mobilization (Simontacchi *et al*. 2012). Furthermore, supplied Fe significantly increased the total Fe in the peanut plants (Table 4), so Fe combined with NO increased content of Fe in cell organelles much more than Fe or NO supplied alone (Table 5).

Nitric oxide can act as antioxidant and break lipid peroxidation. Iron deficiency increased production of reactive oxygen species (ROS) ($O_2^{\bullet -}$ and H_2O_2) and accumulation of MDA, whereas NO and/or Fe supply ameliorated this increase (Fig. 2*D,E,F*). Nitric oxide prevents oxidation damage by regulating cellular redox homeostasis and enhancing H_2O_2-scavenging enzyme activities, and thereby decreases content of H_2O_2 and $O_2^{\bullet -}$ (Shi *et al*. 2007). Besides, simultaneous generations of $O_2^{\bullet -}$ and NO function synergistically in defense responses (Asai *et al*. 2008). Nitric oxide itself can react with $O_2^{\bullet -}$ and generate a peroxynitrite ion ($ONOO^-$). In the physiological pH range, $ONOO^-$ can be protonated and decomposes to a nitrate anion and a proton, or react with H_2O_2 to yield a nitrite anion and oxygen (Wendehenne *et al*. 2001).

Iron deficiency inhibited activities of POD and CAT but stimulated SOD activity (Fig 2*A,B,C*). This may be

because CAT and POD are both heme-containing enzymes, and their activities are tightly linked to content of Fe in plants (Ranieri *et al*. 2001), whereas SOD isoforms contain Cu, Zn, Mn, or Fe as their metal component. In spite of that activity of Fe-SOD decreased, Cu,Zn-SOD activities increased with the increased content of Zn in the Fe deficient plants (Table 4). Previous studies showed similar results in *Pisum sativum* and okra (Jelali *et al*. 2014, Kabir *et al*. 2015). Supplied NO and/or Fe increased activities of antioxidant enzymes, SOD was higher in the alone NO supplied plants than in the Fe supplied plants in contrast to POD and CAT activities. This may be because exogenous NO could alleviate oxidative stress under biotic or abiotic stresses by enhancing the ability of the antioxidant system (Manai *et al*. 2014), and Fe supply significantly increased content of the total Fe in the peanut plants (Table 4). In this study, NO protected peanut against oxidative stress either by reacting with ROS directly or by enhancing activities of ROS-scavenging enzymes.

In plant cells, NO is synthesized by NR (Rockel *et al*. 2002) and NOS (Guo *et al*. 2003). Previous studies have indicated that NO content increases in response to either Fe deprivation or Fe overload suggesting that NO is a key component of regulatory mechanisms that control plant Fe uptake and homeostasis (Arnaud *et al*. 2006, Graziano and Lamattina 2007). Iron deficiency increased NO content in both leaves and roots of peanut, especially after NO supply (Fig. 3*C*), apparently, exogenous NO may affect endogenous NO production. It is consistent with previous reports that NO application significantly increases generation of endogenous NO in tomato and *Arabidopsis* grown under Fe deficiency (Graziano and Lamattina 2007, Chen *et al*. 2010). On the other hand, with the increased NO content, activities of NOS and NR decreased in the NO or NO combined with Fe supplied plants (Fig. 3), whereas in the Fe alone supplied plants, NO content decreased, but activities of NOS and NR increased. This indicates that content of NO could be regulated by activities of NOS and NR and *vice versa*. It was proposed that NO production is increased by an increased Fe content, and a high NO content may induce an increment in root hairs that would contribute to Fe uptake (Martin *et al*. 2009). The inhibition of NO generation appeared after Fe supply, but NO content remained still higher than in the Fe sufficient plants. A similar tendency appeared in NOS and NR activities. This may be because NO could be attained by improving internal Fe availability inside plants (Lee *et al*. 2009), *vice versa*, content of NO in plants may be influenced by increasing content of Fe in plants.

Mineral elements have many functions in plants cells; they are necessary components of proteins and cell walls, act as messengers to signal transduction, participate in electron transport and photosynthesis, *etc*. (Mengel and Kirkby 2001). In this study, the Fe deficient plants contain a greater content of Zn and a lower content of Ca and Mg in young leaves and roots (Table 4); similar results were reported in strawberry and rice (Pestana *et al*. 2012, Pereira *et al*. 2014). Iron transport of iron regulatory protein (IRT) can be also mediated with other divalent cations, such as Cu, Zn, and Mn, uptake (Vert *et al*. 2002) or translocation (Xiong *et al*. 2014). Supplied NO and/or Fe increased content of Ca in shoots and Mg in roots but decreased Zn content in shoots and roots (Table 4). It is possible that NO stimulates Ca^{2+} release from intracellular stores and act as a strong stimulator of Ca^{2+} influx across the PM (Sokolovski and Blatt 2007). The increased content of Fe (Table 4) could balance ion uptake and distribution. Another reason is that NO supply enhanced activity of H^+-ATPase (Fig. 1*A*), and the key membrane enzyme could establish an electrochemical potential gradient to maintain ion balance in plants (Palmgren 2001).

In conclusions, NO and/or Fe supply could change root morphology, increase available Fe and Chl content, regulate NO content, and activities of H^+-ATPase, FCR, NOS, NR, and antioxidant enzymes, as well as distribution of mineral elements. Nitric oxide production and activities of H^+-ATPase and FCR were much more enhanced by NO supply, whereas root length, root/shoot ratio, and root activity were most effectively increased by Fe supply. Obviously, the co-treatment with NO and Fe did the best effects on recovery of peanut chlorosis symptoms, significantly increased content of available Fe and Chl, adjusted Fe and other mineral element distribution in both leaves and roots, and protected peanut from oxidative stress by enhanced activities of antioxidant enzymes.

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