

Leaf responses to iron nutrition and low cadmium in peanut: anatomical properties in relation to gas exchange

Gangrong Shi · Li Sun · Xuming Wang ·
Caifeng Liu

Received: 14 August 2013 / Accepted: 17 October 2013 / Published online: 27 October 2013
© Springer Science+Business Media Dordrecht 2013

Abstract

Aims This study evaluated how iron nutrition affect leaf anatomical and photosynthetic responses to low cadmium and its accumulation in peanut plants.

Methods Seedlings were treated with Cd (0 and 0.2 μM CdCl_2) and Fe (0, 10, 25, 50 or 100 μM $\text{EDTA-Na}_2\text{Fe}$) in hydroponic culture.

Results Cadmium accumulation is highest in Fe-deficient plants, and dramatically decreased with increasing Fe supply. The biomass, gas exchange, and reflectance indices were highest at 25 μM Fe^{2+} treatments, indicating the concentration is favorable for the growth of peanut plants. Both Fe deficiency and Cd exposure impair photosynthesis and reduce reflectance indices. However, they show different effects on leaf anatomical traits. Fe deficiency induces more and smaller stomata in the leaf surface, but does not affect the inner structure. Low Cd results in a thicker lamina with smaller stomata, thicker palisade and spongy tissues, and lower palisade to spongy thickness ratio. The stomatal length and length/width ratio in the upper epidermis, spongy tissue thickness, and palisade to spongy thickness ratio were closely correlated with net photosynthetic rate, stomatal conductance, and transpiration rate.

Conclusions Cd accumulation rather than Fe deficiency alters leaf anatomy that may increase water use efficiency but inhibit photosynthesis.

Keywords Peanut · Cadmium · Iron nutrition · Leaf anatomy · Photosynthesis

Introduction

Cadmium (Cd) is a hazardous metal that is toxic to many plant species at low concentrations. The phytotoxicity of Cd is manifested as causing visual symptoms such as chlorosis, necrosis and wilting, inhibition of plant growth (Sanità di Toppi and Gabbrielli 1999), alteration of structural and ultrastructural (Shi and Cai 2008; Sridhar et al. 2005), reduction of leaf transpiration and photosynthesis (Liu et al. 2011; Mobin and Khan 2007; Shi and Cai 2008), and disturbances in mineral nutrition (Ouariti et al. 1997).

Iron (Fe) is an essential element for plant growth and plays a central role on overall physiology of the plants. Although the total Fe content in soil regularly exceeds plant requirements, it is present as oxihydrates with low bioavailability (Guerinot and Yi 1994), particularly in calcareous soils, which represent 30 % of the earth's surface (Imsande 1998). Fe deficiency causes a range of deleterious effects (e.g. impairing chlorophyll biosynthesis, chloroplast development, and photosynthesis). Additionally, Fe nutrition has been demonstrated to influence on the uptake and accumulation of Cd in plants. Cd efficiently competed with Fe transport via IRT1 and induced Fe deficiency (Su et al. 2013a), so an efficient supply of Fe to plants may decrease Cd uptake (Sarwar et al. 2010). On the other hand, Fe deficiency can induce accelerated Cd accumulation in plants (Rodecap et al. 1994; Su et al. 2013a). Therefore, understanding the effects of Fe

Responsible Editor: Henk Schat.

G. Shi (✉) · L. Sun · X. Wang · C. Liu
College of Life Sciences, Huaibei Normal University,
Huaibei 235000, People's Republic of China
e-mail: swsgr@126.com

nutrition on morpho-physiology and Cd accumulation in plants is very important for crop safe production in soils contaminated with Cd.

Leaves are the major organs of photosynthesis in vascular plants. Leaf structure is closely associated with its physiological function such as photosynthesis and transpiration. Since the uptake and translocation of metals in plants is closely related to the leaf transpiration rate, studying changes in leaf tissues can help understanding the process of metal accumulation (Gomes et al. 2011). Although the Cd-induced alterations of leaf anatomic structure have been investigated in several plant species such as Indian mustard (*Brassica juncea*), peanut (*Arachis hypogaea*), willow (*Salix viminalis*), wheat (*Triticum aestivum*), tomato (*Solanum lycopersicum*), pigeonpea (*Cajanus cajan*), and brachiaria (*Brachiaria decumbens*) (Djebali et al. 2010; Gomes et al. 2011; Khudsar and Iqbal 2001; Kovačević et al. 1999; Shi and Cai 2008; Sridhar et al. 2005; Vollenweider et al. 2006), little is known about the effects of Fe as well as Fe and Cd interaction on the leaf anatomy.

We hypothesize that low Cd and varying Fe supply may induce alteration of leaf anatomical structure in peanut plants, thereby, influence gas exchange. We are interested in the peanut as an experimental crop for several reasons. Firstly, peanut is one of the four major oil crops across the world that is susceptible to Fe deficiency (Zuo and Zhang 2011). Secondly, peanut is often grown in northern China on neutral and calcareous soils, where Fe deficiency is one of the major limiting factors for crop production (Zuo and Zhang 2011). Thirdly, peanut has a higher capability to accumulate Cd in both the vegetative organs and seeds (McLaughlin et al. 2000; Su et al. 2013a, b). Finally, accumulation of Cd in peanut plants is greatly enhanced by Fe deficiency, depending on cultivars (Su et al. 2013a).

The current study was undertaken to test the hypothesis that low Cd and varying Fe supply may induce alteration of leaf anatomical structure in peanut plants, thereby, influence gas exchange. The effects of Fe nutrition on Cd accumulation in peanut plants were also studied.

Materials and methods

Experimental design

Seeds of peanut (*Arachis hypogaea* cv. Haihua 1) were pre-soaked in distilled water for 24 h, and then, they were sown in well washed sand for germination. The 4-day-old seedlings with uniform size were transferred to polyethylene pot (28.5 cm × 18.5 cm × 8.8 cm, three seedlings per pot) filled with 3.5 L of nutrient solution (pH 5.8) (Lu et al. 2013). The nutrient solution was renewed twice a week. After an initial growth period of 14 days under 25 μM EDTA-Na₂Fe conditions, the seedlings were treated with Cd (0 and 0.2 μM CdCl₂) and Fe (0, 10, 25, 50 or 100 μM EDTA-Na₂Fe) in hydroponic culture. The experiment was arranged as a completely random design with three replications (pots). Plants were grown in a growth chamber with a 14-h photoperiod (irradiance of 500 μmol m⁻² s⁻¹), day/night temperatures 28 ± 2/24 ± 1 °C and relative humidity 37 ± 1 %. The pots were randomly moved daily to minimize position effects. Twelve days after Cd and Fe treatments, plants were sampled with leaves to assess the parameters discussed below.

Plant growth and cadmium accumulation

The harvested plants were separated into roots and shoots. Roots were washed with running tap water and soaked in 20 mM Na₂-EDTA for 15 min to remove Cd²⁺ adhering to root surfaces. The roots and shoots were oven-dried for 30 min at 105 °C, and then at 70 °C to a constant weight. The dried tissues were weighed and digested with mixed acid [HNO₃+HClO₄ (3:1, v/v)]. Cd concentration was determined by flame atomic absorbance spectrometry (AAS).

The translocation factor (TF), total Cd in plants and percentage of Cd in shoots were calculated as follows:

$$TF = [Cd]_{\text{shoot}} / [Cd]_{\text{root}}$$

$$\text{Total Cd in plants} = \text{shoot biomass} \times [Cd]_{\text{shoot}} + \text{root biomass} \times [Cd]_{\text{root}}$$

$$\text{Percentage of Cd in shoots} = 100 \times \text{shoot biomass} \times [Cd]_{\text{shoot}} / \text{total Cd in plants}$$

Spectral reflectance measurements

The spectral reflectance of the adaxial surface was measured using a UNISpec spectral analysis system (PP Systems, Haverhill, Massachusetts, USA) over the range of 306–1,138 nm with a 2.0 mm diameter foreoptic and an internal 6.8 W halogen lamp, as the method described by Poulos et al. (2007). Two indices based on the reflectance properties were calculated as follows: (1) a revised version of the normalized difference vegetation index ($_{chl}NDI$) as $_{chl}NDI = (R_{750} - R_{705}) / (R_{750} + R_{705})$ (Gitelson and Merzlyak 1994); (2) the modified red edge simple ratio index (mSR_{705}) as $mSR_{705} = (R_{705} - R_{445}) / (R_{750} - R_{445})$ (Datt 1999).

Gas exchange

Gas exchange parameters including net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) was measured on the highest fully expanded leaf using a portable photosynthesis system (LiCor-6400; Lincoln, NE, USA) equipped with a blue–red light-emitting diode (LED) light source. This experiment was conducted at irradiance of $1,000 \mu mol m^{-2} s^{-1}$, leaf temperature of $25^\circ C$, and CO_2 concentration of $380 \pm 5 \mu mol mol^{-1}$. Water use efficiency (WUE) was calculated as P_n/E .

Leaf anatomical measurements

Sections (5×10 mm) were excised from the middle of the lamina, along with the mid-rib, and fixed in FAA (formaldehyde : acetic acid : 50 % ethanol, 5 : 5 : 90). The samples were dehydrated in an increasing alcohol concentration, embedded in paraffin, sectioned using an ultra microtome ($12 \mu m$ thick) and stained with fast green.

To determine the length, width, and density of stomata apparatus on adaxial and abaxial surfaces, a thin layer of nail polish was applied to the epidermis. Once dry, the nail polish layer was carefully peeled-off with adhesive tape, then fixed on a microscope slide.

All anatomical characteristics were measured using the Image-Pro Express version 6.0 software with an Olympus BX51 light microscope (Olympus, Japan) equipped with a DP71 digital CCD device. Six leaves

per treatment were used for each cultivar, and three separate counts were carried out on each leaf.

Statistical analysis

All data were subject to ANOVA, and statistical significance of the means was compared using Duncan's multiple range test at the 5 % probability level using SPSS software. Relationships between leaf traits were evaluated using Pearson's correlations.

Results

Plant growth

Different Fe treatments affected peanut plant growth (Table 1). The root and shoot biomass were highest in the $25 \mu M Fe^{2+}$ treatments, regardless of the presence or absence of Cd. In contrast to $25 \mu M Fe^{2+}$ treatment, both the Fe deficiency ($0 \mu M Fe^{2+}$) and excess ($100 \mu M Fe^{2+}$) significantly reduced shoot biomass. Root biomass was decreased in Fe excess treatment compared with $25 \mu M Fe^{2+}$ treatment, while it was not changed in Fe deficient treatment, resulting in a higher root /shoot ratio. Cd exposure did not affect the shoot and root biomass as well as the root /shoot ratio. The effects of Fe and Cd interactions on plant growth were not significant.

Cd accumulation and translocation in plants

The accumulation and translocation of Cd in peanut plants were dramatically influenced by Fe nutrition (Table 2). The highest Cd concentrations in roots and shoots were observed in Fe deficient treatment ($0 \mu M Fe^{2+}$), and they were steadily decreased by the increasing of Fe supply. Similar trend was observed in the total Cd in plants. The percentage of Cd in shoots and TF showed a tendency to increase with increasing Fe concentrations in the nutrient solution. There are negative linear correlations between Cd concentration in roots and percentage of Cd in shoots (Fig. 1a), and between total Cd in plants and percentage of Cd in shoots (Fig. 1b). Moreover, asymptotic correlations were also observed between Cd concentration in roots and TF (Fig. 1c), and between total Cd in plants and TF (Fig. 1d).

Table 1 Dry biomass production of peanut plants grown in different Fe nutrition with or without 0.2 μM Cd for 12 d

Treatments	Shoot biomass (g plant^{-1})	Root biomass (g plant^{-1})	Root /shoot ratio
Cd-			
0 $\mu\text{M Fe}^{2+}$	0.88 \pm 0.07 cd	0.23 \pm 0.01 a	0.27 \pm 0.03 a
10 $\mu\text{M Fe}^{2+}$	0.95 \pm 0.05 abc	0.20 \pm 0.01 ab	0.21 \pm 0.00 b
25 $\mu\text{M Fe}^{2+}$	1.08 \pm 0.10 ab	0.23 \pm 0.02 a	0.21 \pm 0.00 b
50 $\mu\text{M Fe}^{2+}$	0.97 \pm 0.02 abc	0.20 \pm 0.02 ab	0.20 \pm 0.01 b
100 $\mu\text{M Fe}^{2+}$	0.91 \pm 0.04 bcd	0.17 \pm 0.01 bc	0.19 \pm 0.01 b
Cd+			
0 $\mu\text{M Fe}^{2+}$	0.80 \pm 0.04 cd	0.21 \pm 0.01 ab	0.26 \pm 0.01 a
10 $\mu\text{M Fe}^{2+}$	0.82 \pm 0.06 cd	0.17 \pm 0.01 bc	0.21 \pm 0.02 b
25 $\mu\text{M Fe}^{2+}$	1.14 \pm 0.04 a	0.23 \pm 0.01 a	0.20 \pm 0.00 b
50 $\mu\text{M Fe}^{2+}$	0.97 \pm 0.09 abc	0.18 \pm 0.01 bc	0.19 \pm 0.02 b
100 $\mu\text{M Fe}^{2+}$	0.75 \pm 0.03 d	0.15 \pm 0.00 c	0.19 \pm 0.00 b
ANOVA			
Cd	2.93 n.s.	2.57 n.s.	0.03 n.s.
Fe	7.62**	11.11***	8.21***
Cd \times Fe	1.13 n.s.	0.55 n.s.	0.13 n.s.

Values (mean \pm SE, $n=3$) with different letters in a column indicate significant differences between treatments at the 0.05 level

ns not significant

** $P<0.01$, *** $P<0.001$

Gas exchange

All the gas exchange parameters including Pn, Gs, Ci, and E were significantly affected by Fe and Cd treatments (Fig. 2). The Pn, Gs, and E were highest in the 25 $\mu\text{M Fe}^{2+}$ treatment, intermediate in 10 and 50 $\mu\text{M Fe}^{2+}$ treatments, and lower in 0 and 100 $\mu\text{M Fe}^{2+}$ treatments; this was more pronounced in the presence of Cd. Exposure to Cd markedly decreased the Pn, Gs, and E. There were significant Cd and Fe interactions on Pn and Ci, indicating Cd-induced changes of Pn and Ci depend on Fe nutrition.

The WUE was considerably affected by Fe and Cd treatments as well as their interaction (Fig. 3). Overall, the WUE was lower in Fe-deficient treatment than other

treatments, particularly in the absent of Cd. Exposure to Cd increased WUE, depending on Fe nutrition.

Spectral reflectance

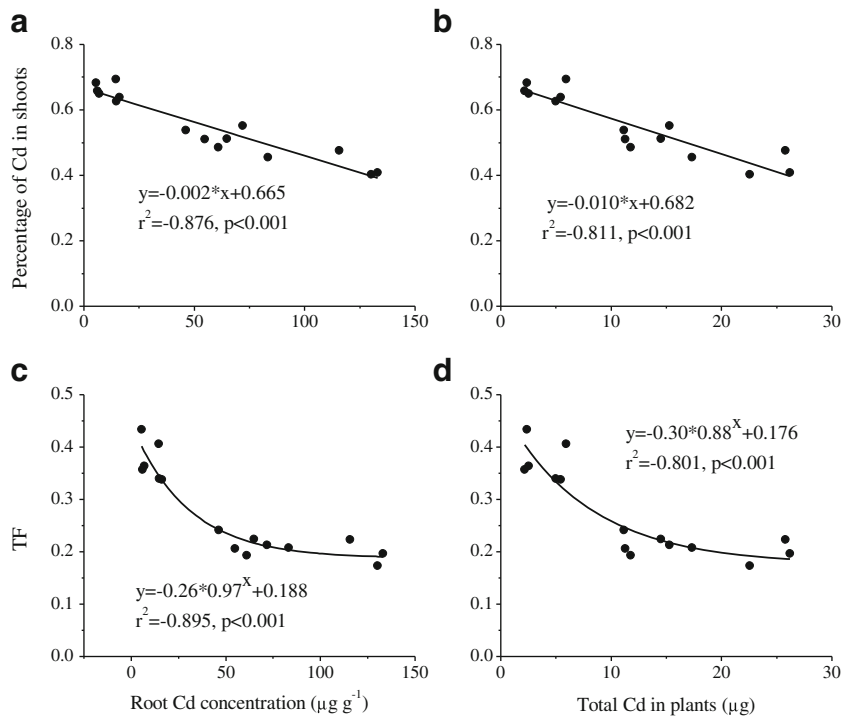
Spectral reflectance was significantly affected by Cd and Fe as well as their interaction could be most clearly seen using a variety of reflectance indices (Fig. 4). By contrast, Fe deficient treatment (0 $\mu\text{M Fe}^{2+}$) showed the lowest mSR₇₀₅ and chlNDI , whereas the highest mSR₇₀₅ and chlNDI were found in the 25 $\mu\text{M Fe}^{2+}$ treatment. Overall, the mSR₇₀₅ and chlNDI were markedly reduced by 0.2 μM Cd, but this is dependent on Fe supply (Fig. 4).

Table 2 Cadmium accumulation and translocation in peanut plants grown in different Fe nutrition with 0.2 μM Cd for 12 d

Treatments	Cd concentration (mg kg^{-1})		Total Cdin plant (μg)	Percentage of Cd in shoots (%)	TF
	Root	Shoot			
0 $\mu\text{M Fe}^{2+}$	126.3 \pm 5.4 a	24.8 \pm 1.1 a	46.2 \pm 1.3 a	0.43 \pm 0.02 c	0.20 \pm 0.01 b
10 $\mu\text{M Fe}^{2+}$	73.4 \pm 5.4 b	15.7 \pm 0.8 b	25.2 \pm 0.9 b	0.51 \pm 0.03 b	0.21 \pm 0.00 b
25 $\mu\text{M Fe}^{2+}$	54.0 \pm 4.3 c	11.4 \pm 0.2 c	25.3 \pm 0.6 b	0.51 \pm 0.02 b	0.21 \pm 0.01 b
50 $\mu\text{M Fe}^{2+}$	15.0 \pm 1.3 d	5.5 \pm 0.3 d	8.1 \pm 0.2 c	0.65 \pm 0.02 a	0.37 \pm 0.05 a
100 $\mu\text{M Fe}^{2+}$	6.2 \pm 0.4 d	2.4 \pm 0.1 e	2.7 \pm 0.0 d	0.66 \pm 0.01 a	0.39 \pm 0.02 a

Values (mean \pm SE, $n=3$) with different letters in a column indicate significant differences between treatments at the 0.05 level

Fig. 1 Relationships between Cd concentration in roots (a, c) and total Cd in plants (b, d) and the percentage of Cd in shoots and TF

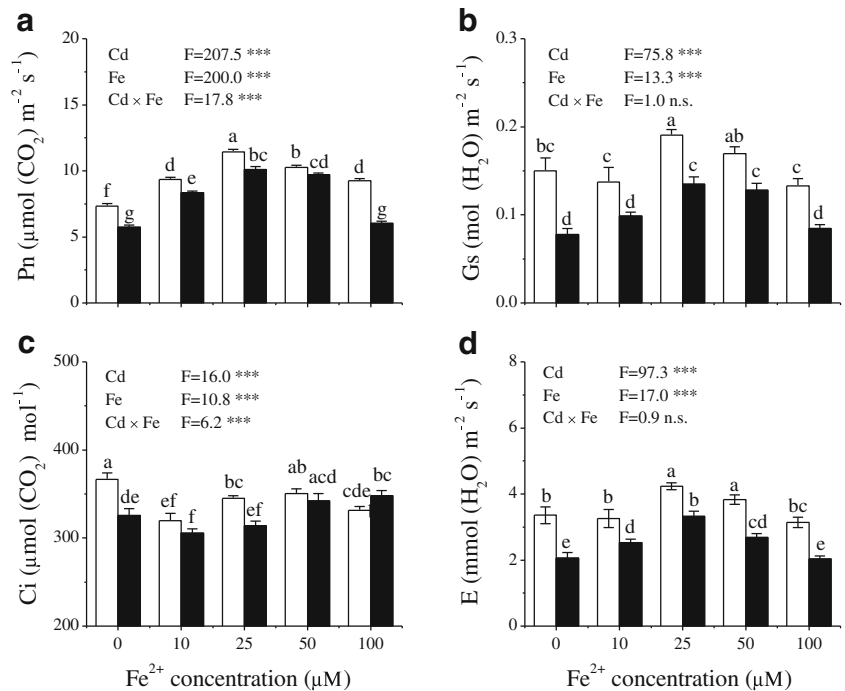


Stomatal characteristics in peanut leaves

Exposure to Cd did not affect the stomatal densities in both the adaxial and abaxial leaf side (Fig. 5), but

appeared to change the size of stomatal apparatus (Table 3). Cd induced decreases in the length of the stomatal apparatus, but enhanced their width, resulting in a lower length /width ratio (Table 3).

Fig. 2 Net photosynthetic rate (a), stomatal conductance (b), intercellular CO_2 concentration (c), and transpiration rate (d) of peanut plants grown in different Fe nutrition with (black bars) or without (white bars) $0.2 \mu\text{M}$ Cd for 12 d. Different letters above the error bars indicate values (mean \pm SE, $n = 18$) significantly different between treatments at the 0.05 level. ns not significant, *** $P < 0.001$



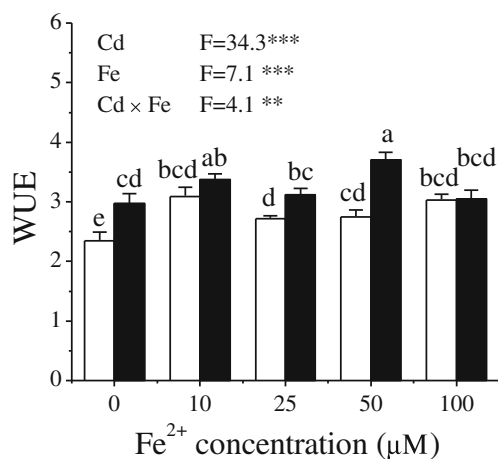


Fig. 3 Water use efficiency (WUE) of peanut plants grown in different Fe nutrition with (black bars) or without (white bars) 0.2 µM Cd for 12 d. Different letters above the error bars indicate values (mean±SE, $n=18$) significantly different between treatments at the 0.05 level. ** $P<0.01$, *** $P<0.001$

Iron nutrition affected the stomatal characteristics including densities, length, width, and length /width ratio of the abaxial and adaxial leaf surface (Table 3, Fig. 5). Compared with 25 µM Fe²⁺ treatment, in which the plants had the longest stomatal apparatus, Fe deficiency (0 µM Fe²⁺) caused more and smaller stomatal apparatus in both the abaxial and adaxial leaf surface.

Leaf anatomical structure

The internal leaf structure of peanut leaves was affected by low Cd treatment (Table 4). Compared with the plants grown in nutrient solution without Cd, the Cd-exposed plants showed thicker leaves with thicker palisade and spongy tissues, smaller palisade to spongy thickness ratio, and thinner upper epidermis. Despite Fe nutrition did not alter the leaf anatomic structure except the thickness of the upper epidermis, there were significant Fe and Cd interactions on the thickness of lamina, upper epidermis, lower epidermis, and palisade tissue.

Relationships between gas exchange, reflectance indices and anatomical properties

From Table 5, one can see that the three gas exchange parameters, Pn, Gs and E, were positively correlated

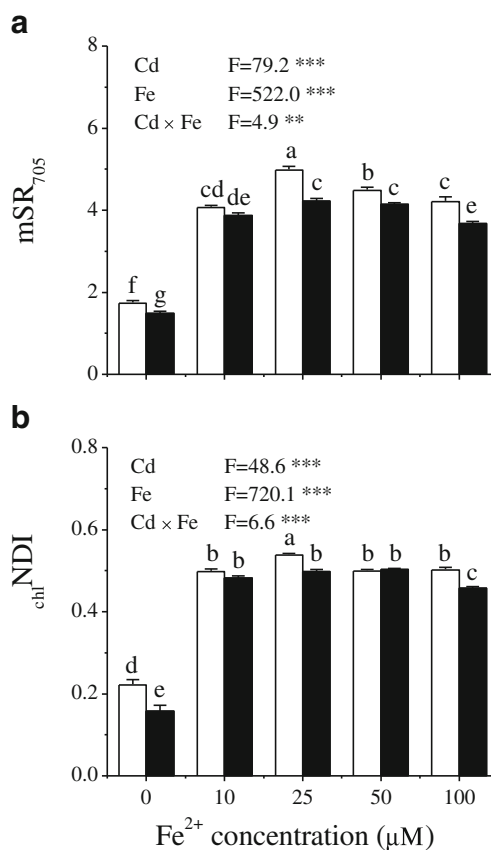


Fig. 4 Modified red edge simple ratio index (a) and normalized difference vegetation index (b) of peanut plants grown in different Fe nutrition with (black bars) or without (white bars) 0.2 µM Cd for 12 d. Different letters above the error bars indicate values (mean±SE, $n=18$) significantly different between treatments at the 0.05 level. ** $P<0.01$, *** $P<0.001$

with each other, whereas they did not significantly relate to Ci. There were positive correlations between mSR₇₀₅ and chlNDI. These parameters were also found to positively correlated with Pn (Table 5).

The results presented in Table 6 showed that the Pn was positively associated with the length and length /width ratio of stomatal apparatus in upper epidermis, and palisade to spongy thickness ratio, but negatively correlated with the stomatal density in lower epidermis and spongy tissue thickness (Table 6). Both the Gs and E were observed to positively correlated with the length and length /width ratio of stomatal apparatus in upper epidermis, upper epidermis thickness, and palisade to spongy thickness ratio, while negatively correlated with the lamina thickness and spongy tissue thickness (Table 6).

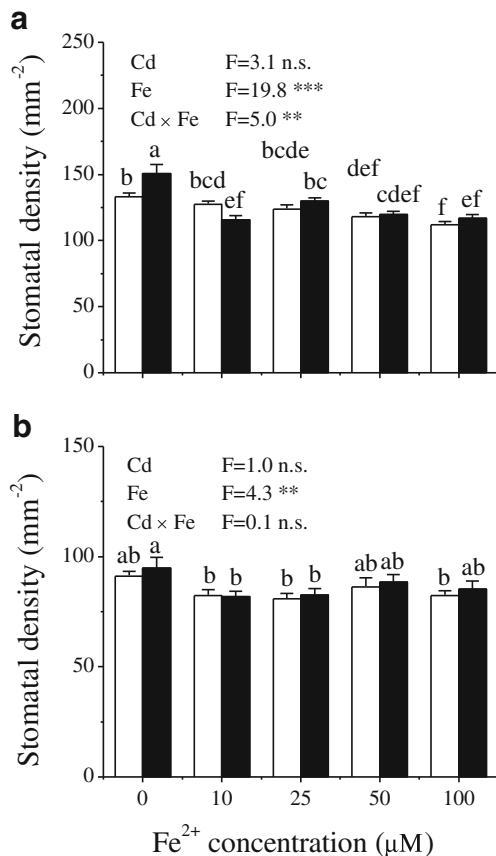


Fig. 5 Stomatal density in adaxial (**a**) and abaxial (**b**) leaf surface of peanut plants grown in different Fe nutrition with (black bars) or without (white bars) 0.2 μM Cd for 12 d. Different letters above the error bars indicate values (mean±SE, $n=12$) significantly different between treatments at the 0.05 level. *ns* not significant, ** $P<0.01$, *** $P<0.001$

Discussion

Effects of Fe nutrition on Cd accumulation in peanut plants

Interactions between Cd and Fe have attracted considerable attention, and antagonistic effects between the two elements have often been reported (Cohen et al. 1998; Martin et al. 2012; Shao et al. 2007; Su et al. 2013a). As expected, our results indicated that Cd uptake and its accumulation in peanut plants are dependent on Fe supply in the nutrient medium. By contrast, Fe-deficient plants showed the highest Cd concentration in roots and shoots as well as total Cd in plants (Table 2). This finding is consistent

with that reported in the literatures (Astolfi et al. 2012; Siedlecka and Krupa 1996). Interestingly, we found that Cd accumulation in peanut plants was steadily decreased by the increasing of Fe supply. The present data appear to indicate that there may be a competition between Cd and Fe absorption in peanut roots. Indeed, symplastic absorption of Cd efficiently competed with Fe for limited specificity of Fe²⁺ transporters such as IRT1 has been reported in both the Strategy I (all plants except graminaceous monocots) (Cohen et al. 1998; Connolly et al. 2002; Eide et al. 1996; Ueno et al. 2008) and Strategy II plants (graminaceous monocots) (Astolfi et al. 2012; Nakanishi et al. 2006).

Different results were reported on influence of external Fe supply on translocation of Cd within plants. For instance, Siedlecka and Krupa (1996) suggested that Fe deficiency result in increase translocation of Cd to leaves, while excessive Fe supply enhances Cd immobilization in roots of pea. In contrast, Su et al. (2013a) found that Fe deficiency causes a reduction in Cd translocation to shoots despite it substantially increases Cd absorption and accumulation in peanut plants. In the present study, both the *TF* and percentage of Cd in shoots showed a tendency to increase with increasing Fe concentrations in the nutrient medium. The results were consistent with our previous findings (Su et al. 2013a), but quite opposite to those of Siedlecka and Krupa (1996). The negative linear relationship between Cd accumulation (Cd concentration in roots and total Cd in plants) and percentage of Cd in shoots (Fig. 1a and b) indicates that more Cd take-up by roots, less proportion of Cd translocate to shoots. This, together with the exponential relation between the Cd accumulation (Cd concentration in roots and total Cd in plants) and *TF* (Fig. 1c and d), suggests that the root-to-shoot transport may be saturated by the increased Cd absorption induced by decreasing Fe supply.

The results obtained in this study appear to be useful for safe crop production with low Cd content. Firstly, the fact that Fe deficiency substantially increase Cd absorption and accumulation provides evidence for the assumption that, when the Fe-deficient soils be polluted by Cd, more risks would pose to human health (Su et al. 2013a). Secondly, Fe fertilization may be a promising technology to control Cd accumulation in crops grown in Cd-polluted soil, because high Fe nutrition was demonstrated to reduce Cd uptake and accumulation in plants by competing with Cd limited common

Table 3 Stomatal size in adaxial and abaxial leaf surface of peanut plants grown in different Fe nutrition with or without 0.2 μM Cd for 12 d

Treatments	Adaxial surface			Abaxial surface		
	Length (μm)	Width (μm)	Length /width ratio	Length (μm)	Width (μm)	Length /width ratio
Cd–						
0 μM Fe ²⁺	26.3±0.2 b	12.5±0.2 bc	2.12±0.03 bcd	31.5±0.3 bc	16.6±0.3 bc	1.91±0.02 b
10 μM Fe ²⁺	26.4±0.2 b	12.4±0.2 bc	2.16±0.04 abc	33.0±0.4 a	16.4±0.2 cd	2.03±0.04 ab
25 μM Fe ²⁺	27.4±0.2 a	12.3±0.2 c	2.26±0.04 a	32.9±0.2 a	16.4±0.3 cd	2.04±0.03 ab
50 μM Fe ²⁺	27.4±0.2 a	12.6±0.2 bc	2.21±0.03 ab	31.2±0.3 bc	15.3±0.2 e	2.07±0.04 a
100 μM Fe ²⁺	27.2±0.3 a	12.6±0.2 bc	2.19±0.05 ab	31.2±0.3 bc	15.8±0.3 cde	2.01±0.04 ab
Cd+						
0 μM Fe ²⁺	24.0±0.2 c	12.7±0.2 bc	1.92±0.04 f	30.7±0.3 c	17.3±0.3 ab	1.80±0.04 c
10 μM Fe ²⁺	26.4±0.2 b	13.5±0.3 a	2.00±0.04 def	31.8±0.3 b	17.9±0.3 a	1.80±0.03 c
25 μM Fe ²⁺	27.7±0.2 a	13.6±0.2 a	2.07±0.04 cde	32.0±0.3 b	16.5±0.3 bcd	1.98±0.04 ab
50 μM Fe ²⁺	26.0±0.2 b	13.5±0.2 a	1.96±0.03 ef	31.3±0.3 bc	15.7±0.3 de	2.02±0.04 ab
100 μM Fe ²⁺	25.9±0.2 b	13.0±0.2 ab	2.02±0.04 def	31.6±0.2 bc	15.9±0.3 cde	2.01±0.03 ab
ANOVA						
Cd	45.0***	31.8***	58.2***	5.8*	10.6**	13.4***
Fe	30.0***	1.2 n.s.	3.6*	8.8***	13.2***	8.3***
Cd×Fe	11.4***	2.3 n.s.	0.4 n.s.	2.7*	2.4 n.s.	2.9*

Values (mean±SE, $n=54$) with different letters in a column indicate significant differences between treatments at the 0.05 level. ns: not significant

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

transporter and/or inhibiting their expression. Shao et al. (2008) showed that soil application of Fe fertilizer (EDTA–Na₂Fe) significantly reduced Cd concentration in rice grain, shoots and roots. Additionally, it may be possible to develop low-Cd transgenic varieties by genetically modifying the Fe transporter gene (Shao et al. 2007).

Effects of Fe nutrition on leaf morpho-physiology of peanut plants

The data presented here show that seedlings treated with 25 μM Fe²⁺ had the highest biomass, efficient photosynthesis as indicated by gas exchange parameters (Pn, Gs and E), and highest mSR₇₀₅ and chlNDI. Because the mSR₇₀₅ were effective in describing changes in chlorophyll content, and chlNDI is usually correlated with chlorophyll a concentration (Liu et al. 2011; Sims and Gamon 2002), the highest mSR₇₀₅ and chlNDI represent a highest chlorophyll content in plant leaves. These data indicate Fe applied at 25 μM Fe²⁺ is the most favorable to the growth of peanut plants.

In contrast to the normal Fe condition (25 μM Fe²⁺), we found that Fe deficiency (0 μM Fe²⁺) significantly inhibits shoot growth and impairs photosynthesis. Analogous results have been reported by several authors (Fernández et al. 2008; Larbi et al. 2006). The decrease of Pn in Fe-deficient plants was accompanied with a decrease in Gs and E (Fig. 2). These results, as suggested by Larbi et al. (2006), indicate that the decrease in Pn induced by Fe deficiency in peanut plants may be attributed to decreases in stomatal opening and transpiration rates. However, the increased Ci under Fe deficiency (Fig. 2c) suggests some non-stomatal factors are also involved in the inhibition of photosynthesis by Fe deficiency (Larbi et al. 2006). Indeed, significant decreases in mSR₇₀₅ and chlNDI were observed in Fe-deficient plants. Moreover, these parameters were also observed to positively correlated with Pn. Thus, the reduction of photosynthetic rate may also result from the decrease in chlorophyll content in Fe-deficient peanut plants.

The effects of Fe deficiency on the leaf stomatal characteristics have not been extensively investigated

Table 4 Leaf inner anatomical traits of peanut plants grown in different Fe nutrition with or without 0.2 μM Cd for 12 d

Treatments	Lamina thickness (μm)	Upper epidermis thickness (μm)	Lower epidermis thickness (μm)	Palisade tissue thickness (μm)	Spongy tissue thickness (μm)	Palisade to spongy thickness ratio
Cd-						
0 μM Fe ²⁺	204±2 cd	15.8±0.5 ab	13.9±0.4 b	83.1±1.8 cd	91.5±1.9 ef	0.93±0.04 a
10 μM Fe ²⁺	197±2 d	14.0±0.4 cd	13.9±0.4 b	80.4±1.3 d	89.1±1.9 f	0.92±0.03 ab
25 μM Fe ²⁺	205±2 c	16.7±0.6 a	15.5±0.4 a	82.8±1.4 cd	90.7±2.4 ef	0.94±0.04 a
50 μM Fe ²⁺	202±3 cd	15.3±0.5 abc	13.8±0.3 b	82.9±1.4 cd	90.4±2.6 ef	0.94±0.03 a
100 μM Fe ²⁺	207±2 bc	15.4±0.6 abc	13.4±0.3 b	84.7±1.6 bcd	93.6±2.2 def	0.93±0.03 ab
Cd+						
0 μM Fe ²⁺	221±4 a	13.2±0.5 d	14.2±0.6 b	85.3±1.8 bcd	107.2±2.8 a	0.81±0.02 c
10 μM Fe ²⁺	219±2 a	14.4±0.4 bcd	14.2±0.4 b	86.0±1.4 abc	104.1±1.7 ab	0.84±0.02 bc
25 μM Fe ²⁺	215±2 a	14.5±0.6 bcd	13.9±0.4 b	88.3±1.8 ab	98.2±2.1 bcd	0.92±0.03 ab
50 μM Fe ²⁺	215±2 a	14.5±0.4 bcd	13.8±0.4 b	90.5±1.7 a	96.6±2.1 cde	0.96±0.03 a
100 μM Fe ²⁺	214±3 ab	15.3±0.3 abc	14.4±0.3 b	82.4±1.7 cd	102.0±2.0 abc	0.82±0.03 c
ANOVA						
Cd	71.5***	11.4***	0.0 n.s.	13.9***	57.7***	10.4**
Fe	0.9 n.s.	2.7 *	1.6 n.s.	1.7 n.s.	2.3 n.s.	2.8 n.s.
Cd×Fe	2.8*	3.4 *	2.9 *	3.0 *	2.0 n.s.	1.8 n.s.

Values (mean±SE, $n=36$) with different letters in a column indicate significant differences between treatments at the 0.05 level
n.s. not significant

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

so far, despite the fact that it is the limiting barrier for the exchange during photosynthesis (Büssis et al. 2006). Fernández et al. (2008) found that, in pear and peach, Fe chlorotic leaves had reductions in the size of guard cells as compared to Fe-sufficient ones, while stomatal densities were not significantly affected by chlorosis. The results obtained in this study showed that, compared with the normal Fe condition (25 μM Fe²⁺ treatment), Fe deficiency (0 μM Fe²⁺) caused an increase in stomatal density, but a decrease in stomatal length in both the abaxial and adaxial leaf surface. The reduction in the stomatal size possibly as a result of the

reduction in leaf growth and expansion processes due to Fe shortage (Shimshi 1967). According to Fernández et al. (2008), final stomatal densities can be affected by disturbances both in differentiation and expansion processes. They suggest that Fe shortage may affect stomatal differentiation, because in Fe chlorotic pear and peach leaves, leaf expansion and the absolute number of stomata per leaf was reduced, whereas stomatal density was not changed significantly. However, in the present study, increased stomatal densities in leaves of Fe-deficient peanut plants indicate that Fe deficiency may also hinder leaf expansion processes.

Smaller stomata which show greater membrane surface area to volume ratio, may have faster response times compared with larger stomata (Drake et al. 2013). Smaller stomata in combine with high density may allow the leaf to attain high operating stomatal conductance rapidly under favorable conditions, and to reduce conductance rapidly when conditions are unfavorable (Drake et al. 2013). Such a stomatal feature usually results in a high WUE, and is considered as a xerophytic characteristics of leaves (Shi and Cai 2009). In the present study, we found that,

Table 5 Correlations ($n=10$) between gas exchange parameters and reflectance indices

	Gs	Ci	E	mSR ₇₀₅	chlNDI
Pn	0.83**	-0.07	0.83**	0.81**	0.74*
Gs		0.41	0.98**	0.50	0.41
Ci			0.27	-0.19	-0.23
E				0.51	0.41
mSR ₇₀₅					0.98**

* $P<0.05$, ** $P<0.01$

Table 6 Correlations ($n=10$) between gas exchange parameters and leaf anatomical features

	Pn	Gs	Ci	E
Stomatal length in upper epidermis	0.82**	0.75*	0.05	0.78**
Stomatal width in upper epidermis	-0.05	-0.45	-0.46	-0.46
Length/width ratio of stomatal apparatus in upper epidermis	0.66*	0.85**	0.3	0.88**
Stomatal length in lower epidermis	0.53	0.48	-0.17	0.52
Stomatal width in lower epidermis	-0.37	-0.45	-0.57	-0.36
Length/width ratio of stomatal apparatus in low epidermis	0.6	0.63	0.42	0.56
Stomatal density in upper epidermis	-0.42	-0.24	-0.04	-0.2
Stomatal density in lower epidermis	-0.64*	-0.37	0.39	-0.44
Lamina thickness	-0.44	-0.71*	-0.4	-0.70*
Upper epidermis thickness	0.44	0.71*	0.63	0.66*
Lower epidermis thickness	0.15	0.24	0.11	0.24
Palisade tissue thickness	0.12	-0.22	-0.28	-0.27
Spongy tissue thickness	-0.67*	-0.88**	-0.44	-0.84**
Palisade to spongy thickness ratio	0.80**	0.86**	0.37	0.79**

* $P<0.05$, ** $P<0.01$

despite the Fe-deficient leaves have smaller and abundant stomata, they show relatively low WUE compared Fe-sufficient ones (Fig. 3). The results obtained in this study provide evidence for the hypothesis that although Fe chlorosis affect stomatal behavior, cuticular rather than stomatal factors could be responsible for the more pronounced water loss (Anderson 1984; Fernández et al. 2008). The reduction of abaxial cuticular weight per unit surface induced by Fe deficiency has been reported by several authors (Anderson 1984; Fernández et al. 2008).

Several studies have focused on the effects of Fe deficiency on leaf anatomical features. Maldonado-Torres et al. (2006) suggested that chlorotic leaves were thicker than green ones, due to increases in the palisade and spongy parenchyma cell length and thickness. In contrast, no significant differences regarding leaf thickness were found between Fe-sufficient and Fe-deficient leaves of pear and peach (Fernández et al. 2008; Morales et al. 1998). Our results, in accordance with the findings on pear and peach (Fernández et al. 2008; Morales et al. 1998), showed that neither leaf thickness nor palisade and spongy tissue thickness were affected by Fe nutrition status. It seems that the inner anatomical structure exhibits less plasticity in response to varying Fe nutrition.

Although there were no major differences in chlNDI and anatomical traits of the Fe excess (100 $\mu\text{M Fe}^{2+}$ treatment) leaves compared to those under normal Fe condition (25 $\mu\text{M Fe}^{2+}$ treatment), Fe excess clearly had

a dramatic effect on plant growth and photosynthesis. The reduction in Pn by Fe excess was associated with the decreasing of Gs and E, while the Ci remain unchanged or even increased in Cd-exposed plants. These results indicated that, apart from stomatal limitation, Fe excess also induces non-stomatal mechanisms that may cause an inhibition in photosynthesis. According to Kampfenkel et al. (1995), Fe excess can generate oxidative stress that may cause photoinhibition, increase the reduction of photosystem II, and enhance thylakoid energization, consequently, result in an inhibition of photosynthesis.

Effects of Cd on leaf morpho-physiology of peanut plants

Although large amount of studies have been carried out for determining the influences of Cd on leaf morpho-physiology of plants including peanut, this study firstly investigated the effects of low Cd on leaf anatomy in relation to gas exchange under different Fe status. Our results showed that Cd applied at low concentration (0.2 μM) does not affect the root and shoot biomass as well as root/shoot ratio. However, it induces some noteworthy changes in leaf spectral reflectance, gas exchange and anatomical structure, depending on exogenous Fe nutrition.

Our results, in agreement with earlier reports (Liu et al. 2011), showed that low-Cd markedly impairs photosynthetic rate of peanut, in which both stomatal

and non-stomatal limitation are involved. Such conclusion was proven by the following observations: (1) the reduction of Pn was significantly associated with the decreasing of Gs and E (Fig. 2, Table 5), and in some cases (0, 10 and 25 $\mu\text{M Fe}^{2+}$ treatments), Cd-induced decrease in Ci were also observed (Fig. 2). These results suggest that the Cd-induced decrease of Pn may be attributed to stomatal limitation, particularly in Fe-deficient or -sufficient peanut plants. (2) Cd caused a reduction in chlorophyll content as indicated by mSR_{705} and chlNDI (Fig. 4), and these reflectance indices were observed to positively correlated with Pn, indicating that the reduction of chlorophyll content may be responsible for the decrease of photosynthetic rate in Cd-exposed peanut plants.

Gas exchange is regulated by controlling the aperture of the stomatal pore and the number of stomata that form on the epidermis (Hetherington and Woodward 2003). In arid environments, smaller stomata allow a rapid response to water stress, while high densities allow maximization of CO_2 diffusion during optimal photosynthetic conditions (Beaulieu et al. 2008; Hetherington and Woodward 2003). Our result indicates that exposure to low-Cd resulted in a decreases in stomatal length and length /width ratio (Table 3), while the stomatal density was unaffected (Fig. 5). Similar results have been reported by Shi and Cai (2008). There were positive correlations between gas exchange parameters (Pn, Gs and E) and size (length and length /width ratio) of stomatal apparatus in the upper epidermis (Table 6). Therefore, the smaller stomata induced by low Cd may be fast stomatal response but limit gas exchange, resulting in an increase of WUE (Fig. 3).

Furthermore, we found evidence that low-Cd affect gas exchange by modifying leaf anatomical structure, depending on Fe supply. Cd applied at low concentration resulted in a thicker lamina with thicker palisade and spongy tissues, and lower palisade to spongy thickness ratio. The results obtained in this study are in accordance with the earlier findings (Shi and Cai 2008, 2009). By contrast, Cd-induced alterations in leaf anatomy were more pronounced in Fe-deficient plants, while in Fe-excess plants, Cd only increased the thickness of spongy tissues, resulting in a decrease of the palisade to spongy thickness ratio (Table 4). Fe nutrition affects Cd-induced alterations in leaf anatomy may be attributed to the changes of Cd concentration in plant tissues by

Fe supply. This was illustrated by the positive correlations between lamina thickness and Cd concentrations in root ($r=0.93, p<0.05, n=5$) and shoot ($r=0.95, p<0.05, n=5$), and negative correlations between the upper epidermis thickness and Cd concentrations in root ($r=-0.92, p<0.05, n=5$) and shoot ($r=-0.93, p<0.05, n=5$).

There were significant and positive correlation between the lamina thickness and palisade ($r=0.70, p<0.05, n=10$), and between the lamina thickness and spongy thickness ($r=0.78, p<0.01, n=10$), indicating Cd-induced thicker lamina was due to an increased mesophyll tissues, particularly the spongy tissues. An increase in mesophyll thickness presents a greater cell wall area for CO_2 diffusion and so should tend to decrease liquid-phase resistance (Mediavilla et al. 2001). The high palisade to spongy ratio are considered as an adaptation for light capture (Fahn 1982); consequently, the low palisade to spongy ratio might partly result in reduction of utilization of light. Actually, we found that the gas exchange parameters (Pn, Gs and E) positively correlated with the palisade to spongy thickness ratio, but negatively correlated with the spongy thickness (Table 6). Thus, we concluded that leaf anatomical features induced by Cd exposure may increase WUE at the cost of loss of photosynthesis.

Conclusions

The uptake and accumulation of Cd in peanut plants are influenced by Fe supply. Cd accumulation is highest in Fe-deficient plants, and dramatically decreased with increasing Fe supply. Fe nutrient significantly affects plant growth and leaf morphology of peanut. Fe applied at 25 $\mu\text{M Fe}^{2+}$ is the most favorable to the growth of peanut plants. Fe deficiency (0 $\mu\text{M Fe}^{2+}$) causes smaller and abundant stomata in the leaf surface and decreases chlorophyll content as indicated by mSR_{705} and chlNDI , leading to inhibition of photosynthesis. Cd applied at low concentration (0.2 μM) reduces chlorophyll content and results in a thicker lamina with smaller stomata, thicker palisade and spongy tissues, and lower palisade to spongy thickness ratio; this is dependent on Fe supply. Fe-induced alteration in Cd accumulation rather than Fe nutrition itself affects leaf anatomy that may change gas exchange.

Acknowledgments Financial support from the National Natural Science Foundation of China (No. 31171464) and the Anhui Provincial Natural Science Foundation (No. 11040606M87, 1308085MC47) is gratefully acknowledged. We would like to acknowledge the two anonymous reviewers for their helpful comments and suggestions.

References

- Anderson CA (1984) Development of leaf water deficits in detached green and lime-chlorotic leaves of seedlings from populations of *Eucalyptus obliqua* L'Hérit. *Plant Soil* 77: 171–181
- Astolfi S, Zuchi S, Neumann G, Cesco S, Sanità di Toppi L, Pinton R (2012) Response of barley plants to Fe deficiency and Cd contamination as affected by S starvation. *J Exp Bot* 63:1241–1250
- Beaulieu JM, Leitch IJ, Patel S, Pendharker A, Knight CA (2008) Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytol* 179:975–986
- Büßis D, von Groll U, Fisahn J, Altmann T (2006) Stomatal aperture can compensate altered stomatal density in *Arabidopsis thaliana* at growth light conditions. *Funct Plant Biol* 33:1037–1043
- Cohen CK, Fox TC, Garvin DF, Kochian LV (1998) The role of iron-deficiency stress responses in stimulating heavy-metal transport in plants. *Plant Physiol* 116:1063–1072
- Connolly EL, Fett JP, Guerinot ML (2002) Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. *Plant Cell* 14:1347–1357
- Datt B (1999) A new reflectance index for remote sensing of chlorophyll content in higher plants: tests using *Eucalyptus* leaves. *J Plant Physiol* 154:30–36
- Djebali W, Hediji H, Abbes Z, Barhoumi Z, Yaakoubi H, Zoghalmi LB, Chaibi W (2010) Aspects on growth and anatomy of internodes and leaves of cadmium-treated *Solanum lycopersicum* L. plants. *J Biol Res (Thessaloniki)* 13:75–84
- Drake PL, Froend RH, Franks PJ (2013) Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. *J Exp Bot* 64:495–505
- Eide D, Broderius M, Fett J, Guerinot ML (1996) A novel iron-regulated metal transporter from plants identified by functional expression in yeast. *Proc Natl Acad Sci U S A* 93:5624–5628
- Fahn A (1982) *Plant anatomy*. Pergamon Press, New York
- Fernández V, Eichert T, Del Río V, López-Casado G, Heredia-Guerrero JA, Abadía A, Heredia A, Abadía J (2008) Leaf structural changes associated with iron deficiency chlorosis in field-grown pear and peach: physiological implications. *Plant Soil* 311:161–172
- Gitelson A, Merzlyak MN (1994) Spectral reflectance changes associated with autumn senescence of *Aesculus hippocastanum* L. and *Acer platanoides* L. leaves. Spectral features and relation to chlorophyll estimation. *J Plant Physiol* 143:286–292
- Gomes MP, Marques TCLLDM, Nogueira MDG, Castro EM, Soares AM (2011) Ecophysiological and anatomical changes due to uptake and accumulation of heavy metal in *Brachiaria decumbens*. *Sci Agric* 68:566–573
- Guerinot ML, Yi Y (1994) Iron: nutritious, noxious, and not readily available. *Plant Physiol* 104:815–820
- Hetherington AM, Woodward FI (2003) The role of stomata in sensing and driving environmental change. *Nature* 424: 901–908
- Imsande J (1998) Iron, sulfur, and chlorophyll deficiencies: a need for an integrative approach in plant physiology. *Physiol Plant* 103:139–144
- Kampfenkel K, Montagu M, Inzé D (1995) Effects of iron excess on *Nicotiana plumbaginifolia* plants (implications to oxidative stress). *Plant Physiol* 107:725–735
- Khudsar T, Iqbal M (2001) Cadmium-induced changes in leaf epidermes, photosynthetic rate and pigment concentrations in *Cajanus cajan*. *Biol Plant* 44:59–64
- Kovačević G, Kastori R, Merkulov LJ (1999) Dry matter and leaf structure in young wheat plants as affected by cadmium, lead, and nickel. *Biol Plant* 42:119–123
- Larbi A, Abadía A, Abadía J, Morales F (2006) Down co-regulation of light absorption, photochemistry, and carboxylation in Fe-deficient plants growing in different environments. *Photosynth Res* 89:113–126
- Liu C, Guo J, Cui Y, Lü T, Zhang X, Shi G (2011) Effects of cadmium and salicylic acid on growth, spectral reflectance and photosynthesis of castor bean seedlings. *Plant Soil* 344: 131–141
- Lu Z, Zhang Z, Su Y, Liu C, Shi G (2013) Cultivar variation in morphological response of peanut roots to cadmium stress and its relation to cadmium accumulation. *Ecotoxicol Environ Saf* 91:147–155
- Maldonado-Torres R, Etchevers-Barra JD, Alcántar-González G, Rodríguez-Alcazar J, Colinas-León MT (2006) Morphological changes in leaves of Mexican lime affected by iron chlorosis. *J Plant Nutr* 29:615–628
- Martin SR, Llugany M, Barceló J, Poschenrieder C (2012) Cadmium exclusion a key factor in differential Cd-resistance in *Thlaspi arvense* ecotypes. *Biol Plant* 56:729–734
- McLaughlin MJ, Bell MJ, Wright GC, Cozens GD (2000) Uptake and partitioning of cadmium by cultivars of peanut (*Arachis hypogaea* L.). *Plant Soil* 222:51–58
- Mediavilla S, Escudero A, Heilmeyer H (2001) Internal leaf anatomy and photosynthetic resource-use efficiency: interspecific and intraspecific comparisons. *Tree Physiol* 21:251–259
- Mobin M, Khan NA (2007) Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. *J Plant Physiol* 164: 601–610
- Morales F, Grasa R, Abadía A, Abadía J (1998) Iron chlorosis paradox in fruit trees. *J Plant Nutr* 21:815–825
- Nakanishi H, Ogawa I, Ishimaru Y, Mori S, Nishizawa NK (2006) Iron deficiency enhances cadmium uptake and translocation mediated by the Fe²⁺ transporters OsIRT1 and OsIRT2 in rice. *Soil Sci Plant Nutr* 52:464–469
- Ouariti O, Gouia H, Ghorbal MH (1997) Responses of bean and tomato plants to cadmium: growth, mineral nutrition, and nitrate reduction. *Plant Physiol Biochem* 35:347–354
- Poulos HM, Goodale UM, Berlyn GP (2007) Drought response of two Mexican oak species, *Quercus laceyi* and *Q.*

- sideroxylo* (Fagaceae), in relation to elevational position. *Am J Bot* 94:809–818
- Rodecap KD, Tingey DT, Lee EH (1994) Iron nutrition influence on cadmium accumulation by *Arabidopsis thaliana* (L.) Heynh. *J Environ Qual* 23:239–246
- Sanità di Toppi L, Gabbriellini R (1999) Response to cadmium in higher plants. *Environ Exp Bot* 41:105–130
- Sarwar N, Malhi SS, Zia MH, Naeem A, Bibi S, Farid G (2010) Role of mineral nutrition in minimizing cadmium accumulation by plants. *J Sci Food Agric* 90:925–937
- Shao G, Chen M, Wang W, Mou R, Zhang G (2007) Iron nutrition affects cadmium accumulation and toxicity in rice plants. *Plant Growth Regul* 53:33–42
- Shao G, Chen M, Wang D, Xu C, Mou R, Cao Z, Zhang X (2008) Using iron fertilizer to control Cd accumulation in rice plants: a new promising technology. *Sci China C Life Sci* 51:245–253
- Shi G, Cai Q (2008) Photosynthetic and anatomic responses of peanut leaves to cadmium stress. *Photosynthetica* 46:627–630
- Shi G, Cai Q (2009) Leaf plasticity in peanut (*Arachis hypogaea* L.) in response to heavy metal stress. *Environ Exp Bot* 67: 112–117
- Shimshi D (1967) Leaf chlorosis and stomatal aperture. *New Phytol* 66:455–461
- Siedlecka A, Krupa Z (1996) Interaction between cadmium and iron and its effects on photosynthetic capacity of primary leaves of *Phaseolus vulgaris*. *Plant Physiol Biochem* 34: 833–841
- Sims DA, Gamon JA (2002) Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sens Environ* 81:337–354
- Sridhar BB, Diehl SV, Han FX, Monts DL, Su Y (2005) Anatomical changes due to uptake and accumulation of Zn and Cd in Indian mustard (*Brassica juncea*). *Environ Exp Bot* 54:131–141
- Su G, Li F, Lin J, Liu C, Shi G (2013a) Peanut as a potential crop for bioenergy production via Cd-phytoextraction: a life-cycle pot experiment. *Plant Soil* 365:337–345
- Su Y, Wang X, Liu C, Shi G (2013b) Variation in cadmium accumulation and translocation among peanut cultivars as affected by iron deficiency. *Plant Soil* 363:201–213
- Ueno D, Iwashita T, Zhao FJ, Ma JF (2008) Characterization of Cd translocation and identification of the Cd form in xylem sap of the Cd-hyperaccumulator *Arabidopsis halleri*. *Plant Cell Physiol* 49:540–548
- Vollenweider P, Cosio C, Günthardt-Goerg MS, Keller C (2006) Localization and effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.): Part II Microlocalization and cellular effects of cadmium. *Environ Exp Bot* 58:25–40
- Zuo Y, Zhang F (2011) Soil and crop management strategies to prevent iron deficiency in crops. *Plant Soil* 339:83–95