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Effects of iron deficiency on subcellular distribution and chemical forms of cadmium in peanut roots in relation to its translocation

Ying Su, Junli Liu, Ziwei Lu, Xuming Wang, Zheng Zhang, Gangrong Shi*

College of Life Sciences, Huaibei Normal University, Huaibei 235000, PR China

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ABSTRACT

Effects of iron deficiency on subcellular distribution and chemical forms of Cd in four peanut (*Arachis hypogaea* L.) cultivars were investigated by a hydroponics experiment, at low Cd level (0.2 μM $CdCl_2$). The results show that, compared with high Cd accumulating cultivars, the low Cd accumulating cultivars show higher biomass production, more chlorophyll, and less Cd accumulation in shoots. Higher proportion of Cd in the soluble fraction was also observed in low Cd accumulating cultivars that may contribute to low Cd accumulation in their shoots. Fe deficiency increases Cd uptake and accumulation in plants, but decreases Cd translocation from roots to shoots. It was also observed that Fe deficiency increase the proportion of Cd in the soluble fraction and the proportion of NaCl extractable Cd, which were negatively correlated with shoot Cd concentration. The percentage of NaCl extractable Cd was negatively and exponentially related to the percentage of Cd in shoots and translocation factors of Cd to shoots. It seems that a high proportion of Cd in the soluble fraction (mainly in vacuoles) and a high proportion of NaCl extractable Cd (pectate and protein-bound Cd) are responsible for the decreased Cd translocation by Fe deficiency.

1. Introduction

Cadmium (Cd) is a toxic heavy metal released into the environment by heating systems, metallurgic industries, waste incinerators, urban traffic, cement factories, and as a contaminant of phosphate fertilizers (Gallego et al., 2012). Although Cd is not essential for plant nutrition, it can be easily taken up by roots and accumulated in all plant tissues, from roots to the above-ground organs (Gratão et al., 2008; Tezotto et al., 2012; Vitória et al., 2001). Moreover, the accumulation and translocation may vary within the same plant species depending on the genetic background (Monteiro et al., 2011; Su et al., 2013a). The accumulation of Cd can lead to a state of oxidative stress which can affect dramatically plant growth and metabolism (Gratão et al., 2012). It can also cause some morphological, structural and ultrastructural alterations to plant tissues (Gratão et al., 2009; Shi and Cai, 2008; Vitória et al., 2003; Zhang et al., 2013). Moderate Cd contamination in soils may result in considerable Cd accumulation in the edible parts of crops, posing a serious problem on safe food production (Li et al., 2006). Nevertheless, it is important to take into consideration the soil type and how much of the metal is readily and biological available to the plant roots (Gratão et al., 2005; Melo et al., 2011). Therefore, it is desirable to develop both agronomic management practices and breeding strategies to reduce Cd accumulation in edible parts of plants (Nocito et al., 2011; Souza et al., 2013). Toward these aims, a prior understanding of the basic mechanisms by which Cd is transported is required.

There are two transport processes most likely to mediate Cd accumulation into the shoots: (i) uptake and sequestration in roots, and (ii) xylem-loading-mediated translocation to shoots (Clemens et al., 2002). The absorption in roots has been considered a key process in overall plant Cd accumulation (Uraguchi et al., 2009). As a non-essential element, Cd is loaded from the symplasm into the xylem by uptake systems for essential elements such as Fe, Ca and Zn (Clemens, 2006). Once inside root cells, Cd ions have to be trapped inside cells by the formation of metal-chelating molecules and transporter-mediated vacuolar sequestration (Clemens, 2006; Clemens et al., 2002). This process may restrict Cd delivery to the xylem from the symplast, and contribute to the natural variation in Cd distribution between roots and shoots observed in crop species (Nocito et al., 2011).

It is well known that Cd efficiently competed with Fe transport via IRT1 and induced Fe deficiency (Astolfi et al., 2012; Connolly et al., 2002; Nakanishi et al., 2006), so an efficient supply of Fe to plants may decrease Cd uptake (Sarwar et al., 2010). Numerous studies have demonstrated that Fe deficiency can considerably increase Cd accumulation in plants (Astolfi et al., 2012; Connolly et al., 2002; Nakanishi et al., 2006; Su et al., 2013a). In a previous study, we found that Fe deficiency increased the concentration and amount of Cd in plants for all the twelve peanut (*Arachis hypogaea*

* Tel.: +86 56 1380 2225; fax: +86 56 1380 3237.
E-mail address: swsgr@126.com (G. Shi).

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L.) cultivars tested, but decreased the translocation of Cd from root to shoot. Fe deficiency affects the accumulation and translocation of Cd in a cultivar-dependent manner (Su et al., 2013a). We assumed that the increased root Cd concentration in Fe-deficient plants may result in alterations in subcellular distribution and chemical forms of Cd in roots, leading to an efficient Cd ions trapping system and consequently, limiting the root-to-shoot translocation of Cd. However, little evidence is available for this hypothesis.

The present study aimed to (i) evaluate the influence of Fe deficiency on subcellular distribution and chemical forms of Cd in peanut roots, and (ii) address the hypothesis that these alterations may be responsible for the Fe deficiency-induced decrease in root-to-shoot translocation of Cd in peanut plants.

2. Materials and methods

2.1. Experimental design

According to the previous studies, two low Cd accumulating (Luhua 8, Xvhua 13) and two high Cd accumulating (Haihua 1, Zhenghong 3) cultivars of peanut were selected for hydroponic experiment. Seeds were surface sterilized in 5% sodium hypochlorite for 1 min, and then, they were soaked in distilled water for 24 h and 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) filled with 3.5 L of nutrient solution (pH 5.8) (Lu et al., 2013). After an initial growth period of 14 days, the seedlings were treated with 0 (Fe deficiency) and 50 (Fe sufficiency) μM EDTA-Na_2Fe in hydroponic culture containing 0.2 μM $CdCl_2$. 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}), temperatures of 29 ± 1 °C (day)/25 ± 1 °C (night), and relative humidity of 33 ± 7% (day)/39 ± 4% (night). The pots were randomly moved daily to minimize position effects.

2.2. 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 Na_2-EDTA for 15 min to remove Cd^{2+} 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 [HNO_3 + $HClO_4$ (3:1, v/v)]. Cd concentration was determined by graphite furnace atomic absorption spectrometry (GFAAS).

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

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

Percentage of Cd in shoots = 100 × shoot biomass × $[Cd]_{shoot}$/(shoot biomass × $[Cd]_{shoot}$ + root biomass × $[Cd]_{root}$)

2.3. Tissue fractionation

Frozen root tissues (0.2 g) were ground into powder by adding 10 mL of extraction solution containing 0.25 mM sucrose, 50 mM Tris–HCl buffer solution (pH 7.5), and 1.0 mM DL-dithioerythritol. The homogenate was centrifuged at 3000 r min^{-1} for 15 min and the precipitation was designated as 'cell wall fraction' mainly consisting of cell walls and cell wall debris. The resulting supernatant solution was further centrifuged at 15,000 r min^{-1} for 30 min. The resultant deposition and the supernatant solution were referred to as 'organelle fraction' and 'soluble fraction', respectively. All operations were undertaken at 4 °C. The cell wall and cell organelle fractions were transferred to 100 mL Erlenmeyer conical flasks with de-ionized water, evaporated to dryness, and digested with 5 mL of HNO_3. Cd concentrations of the soluble fraction and digested samples were analyzed by GFAAS.

2.4. Extraction of Cd in different chemical forms

Chemical forms of Cd in peanut roots were successively extracted by designated solutions in the following order: (1) 80% ethanol, extracting inorganic Cd including nitrate/nitrite, chloride and aminophenol cadmium; (2) de-ionized water (d-H_2O), extracting water soluble Cd, Cd-organic acid complexes and $Cd(H_2PO_4)_2$; (3) 1 M NaCl, extracting pectate and protein-integrated Cd; (4) 2% acetic acid (HAc), extracting undissolved cadmium phosphate including $CdHPO_4$ and $Cd_3(PO_4)_2$ and (5) 0.6 M HCl, extracting cadmium oxalate (Fu et al., 2011).

Frozen root tissues (0.5 g) were homogenized in extraction solution (w/v, 1:10) with a mortar and pestle, and shaken for 22 h at 25 °C. Then the homogenate was centrifuged at 5000 × *g* for 10 min, obtaining the first supernatant solution in a conical beaker. Sedimentation was re-suspended twice in the extraction solution, shaken for 2 h at 25 °C, and centrifuged at 5000 × *g* for 10 min. Subsequently the supernatants of the three suspensions and centrifuge steps for each of the five extraction solutions were pooled. Each pooled solution was evaporated on an electric plate at 70 °C to constant weight, and digested with 5 mL of HNO_3-$HClO_4$ (3:1, v/v). Cd concentrations were analyzed by GFAAS.

2.5. Statistical analysis

Data were analyzed by ANOVA using SPSS software (version 13.0). Duncan's test was used to determine the significant differences between means ($p < 0.05$). Relationships between the concentration, translocation, and distribution of Cd in plants were determined by linear or non-linear regression analysis.

3. Results

3.1. Effects of Fe deficiency on biomass production and leaf SPAD values

Peanut differ from each other in dry biomasses among the four cultivars tested (Table 1). The growth parameters in terms of shoot and root biomass and root/shoot ratio, were highest in Luhua 8, and lowest in Haihua 1. By contrast, low Cd accumulating cultivars (Luhua 8 and Xvhua 13) exhibited higher biomass and root/shoot ratio than high Cd accumulating cultivars (Haihua 1 and Zhenghong 3).

Iron deficiency did not affect the shoot biomass, but slightly enhanced root biomass for all cultivars compared with Fe-sufficient treatment, resulting in an increase in root/shoot ratio (Table 1). The interactive effects of cultivar and Fe on biomass production and root/shoot ratio were not significant (Table 1).

Leaf chlorophyll contents as indicated by SPAD values were significantly affected by cultivar and Fe treatments as well as their interaction (Table 1). For Fe-sufficient treatment, chlorophyll contents were considerably higher in the two low Cd accumulating cultivars compared with the two high Cd accumulating cultivars. However, under Fe-deficient conditions, chlorophyll contents were highest in Xvhua 13, intermediate in Haihua 1 and Luhua 8, and lowest in Zhenghong 3. Fe deficiency reduced leaf chlorophyll contents in a cultivar-dependent manner. The declines of chlorophyll contents in Fe-deficient plants were 20.7%, 34.2%, 39.3%, and 42.4% for Xvhua 13, Haihua 1, Luhua 8, and Zhenghong 3, respectively, compared with those of Fe-sufficient ones (Table 1).