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Effects of grafting on root-to-shoot cadmium translocation in plants of eggplant (*Solanum melongena*) and tomato (*Solanum lycopersicum*)

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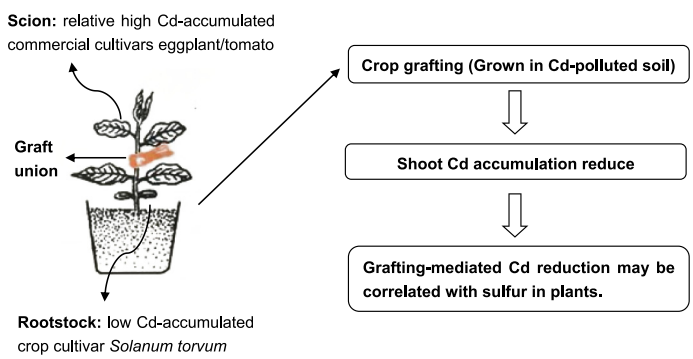
HIGHLIGHTS

- Grafting on *S. torvum* greatly reduced Cd accumulation in eggplant or tomato leaves.
- Self-grafting did not directly redcue Cd accumulation in eggplant or tomato.
- A close positive correlation occurred between Cd and sulfur accumulation in leaves.

GRAPHICAL ABSTRACT



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ABSTRACT

Heavy metal cadmium (Cd) pollution in farmland has become a serious threat to food security globally. In this work, a grafting technique was applied to eggplant (*Solanum melongena*) and tomato (*Solanum lycopersicum*) plants using *Solanum torvum* as rootstock to investigate effects of grafting on Cd accumulation in shoots. The un-grafted, self-grafted, and grafted plants were grown in soils containing 2 mg kg^{-1} Cd. Results showed that grafting on *S. torvum* could efficiently reduce Cd accumulation in leaves of eggplant and tomato, and the decrease was 89% and 72%, respectively. With *S. torvum* as rootstock, Cd concentrations were 1.11 mg kg^{-1} and 6.58 mg kg^{-1} in leaves of grafted eggplant and tomato, which were significantly decreased as compared with un-grafted plants (10.12 mg kg^{-1} and 23.19 mg kg^{-1}, respectively, $p < 0.05$). In addition, Cd concentrations were 12.11 mg kg^{-1} and 29.47 mg kg^{-1} in leaves of self-grafted eggplant and tomato, respectively, which was similar to those in un-grafted eggplant, but more than those in un-grafted tomato ($p < 0.05$). This suggests that the *S. torvum* rootstock, and not the grafting operation, was responsible for efficient reduction of Cd accumulation in shoots of eggplant and tomato plants. Furthermore, total sulfur and sulfate (SO_4^{2-}) concentrations analysis revealed that there was a similar trend between Cd accumulation and total sulfur or SO_4^{2-} concentrations in leaves of plants tested. Additionally, a strong positive correlation between Cd accumulation and total sulfur or SO_4^{2-} concentrations occurred in leaves of eggplant and tomato plants. Thus, sulfur, mainly SO_4^{2-}, in leaves may play an important regulatory role in Cd accumulation of eggplant and tomato plants. This study provides the theoretical and technical support for applying grafting technique for the safe practice of farming in Cd-contaminated agricultural soil.

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1. Introduction

Soil contamination of heavy metals has become a worldwide problem. One of the heavy metals of high concern is cadmium (Cd). Cd has been reported to enter into the agro-ecosystem through both natural and anthropogenic processes, such as weathering of minerals, mining, traffic, wastewater irrigation (Chen et al., 2013; Gu et al., 2014; Jeelani et al., 2017), sewage sludge disposal (Minari et al., 2017), and application of fertilizers and animal manure application (Alloway and Steinnes, 1999; Jiao et al., 2012; Ryan et al., 1982). Cd can be easily absorbed by plant roots when grown in Cd-polluted soil, this Cd can then be transferred from roots to shoots, and finally accumulated in the edible parts of the plant. This absorption of Cd not only inhibits plant growth and development, but also has the potential to affect human health (López-Millán et al., 2009). Therefore, reducing Cd uptake by crops or preventing the root-to-shoot translocation is an important agronomic management strategy for the safe use of slightly to intermediately contaminated arable land.

Various approaches have been proposed to reduce Cd accumulation in plants grown on Cd contaminated land. One of these approaches is to "deactivate" Cd in soil, i.e. to reduce Cd bioavailability via measures such as increasing soil pH, applying adsorbents, and keeping fields flooded for longer durations (Arao et al., 2009; Cattani et al., 2008; Chen et al., 2017; Kirkham, 2006; Qian et al., 2017). Another means is to "clean-up" the contaminated soil simultaneously with agricultural production via co-planting of Cd-hyperaccumulator plants and then removing these plant residuals (Li et al., 2018; Rosenfeld et al., 2018; Wu et al., 2006). A third way to reduce Cd concentrations in agricultural products is to select or breed low-accumulating crops in these areas (Wang et al., 2011; Yang et al., 2017). However, cost effectiveness and/or environmental pressure have limited the widespread use of these methods. Alternatively, grafting has the potential to regulate heavy metal accumulation in the edible parts of crops. Grafting is a physical combination of two or more pieces of living plant tissues through forced vascular connection resulting in a single plant. Grafting is not only a means of vegetative propagation in botanical horticulture, but also is still widely used to mitigate plant stresses caused by adverse conditions, such as heavy metal pollution (Savvas et al., 2010). The levels of Cd uptake and accumulation vary significantly in different species of plants, or among cultivars of the same plant species (Kuboi et al., 1986). Increasing evidence has demonstrated that grafting can effectively reduce the accumulation of heavy metals in the shoots of crops. For instance, grafting on *S. torvum* rootstock led to a reduction of Cd concentrations in fruits, leaves, and stems of eggplant (Arao et al., 2008). Grafting onto commercial pumpkin rootstock 'Shintoza' (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne) significantly decreased Cu accumulation in cucumber leaves as compared to the un-grafted cultivars (Rouphael et al., 2008). Although significant decreases in Cd accumulation have been noted with grafting, interestingly, it is still unclear which mechanisms are behind this effect.

One mechanism of these decreases in Cd concentrations could lie in the effect that grafting may have on other compounds in plants. Cd is an extremely reactive heavy metal with an affinity towards some biomolecules such as amino, thiol groups, carboxy (Cefola, 1966). Many studies have shown that S-containing compounds played an important role in Cd accumulation in plants (Liang et al., 2014; Zhang et al., 2012). The effect of grafting on S-containing compounds, as a possible mechanism for the reduction in the accumulation of Cd, has yet to be fully explored. What is currently known is that for sulfur (S) assimilation and metabolism in plants, sulfate (SO_4^{2-}) is absorbed into roots and most of this compound is transferred to the shoots, which then can be reduced to cysteine (Cys), and further synthesized into various metabolites including glutathione (GSH), photochelations (PCs), and methionine (Met) (Long et al., 2002). Furthermore, PCs are likely to be synthesized in plants by the induction of Cd stress. Interestingly, the role of PCs in the detoxification of heavy metals had been more widely focused on by researchers (Zenk, 1996). However, emerging studies have also shown that PCs and GSH may be the 'transporters' responsible for long-distance Cd translocation from roots to shoots (Chen et al., 2006; Gong et al., 2003; Mendoza-Cózatl et al., 2008), making the role of sulfur more unclear. To date, most studies have focused on the response mechanism of sulfur assimilation in regards to Cd stress (Anjum et al., 2008; Harada et al., 2002; Pereira et al., 2002; Zhou et al., 2014), the current study will add to this growing body of literature by also evaluating how sulfur may be effected by grafting.

Eggplant (*Solanum melongena*) and tomato (*Solanum lycopersicum*) are different genera within the same family (*Solanaceae*). These plants are known to have a high Cd accumulation potential (Arthur et al., 2000; Kuboi et al., 1986). In previous studies, Cd concentrations were greatly reduced in grafted eggplant cultivar Senryou2 using *S. torvum* as a rootstock (Arao et al., 2008), thus it is worth evaluating whether *S. torvum* rootstock can be widely used for reducing Cd accumulation in other *Solanaceae* plants. Additionally, tomato was chosen based on its high importance as a vegetable crop worldwide (Cikili et al., 2016; Xu et al., 2018). This is a good representative crop as it is also generally cultivated in areas wherein special substrates and heavy use of feritilizers are required, thereby imposing an increased risk of Cd accumulation (Angelova et al., 2009; Avci and Deveci, 2013; López-Millán et al., 2009). In the present study, the grafting technique was evaluated for two *Solanaceae* plants (eggplant and tomato) using *S. torvum* as rootstock, with the aims (i) to investigate effect of grafting on Cd translocation from roots to shoots in both plants; and (ii) to explore the roles of total sulfur and SO_4^{2-} in Cd accumulation in eggplant and tomato plants.

2. Materials and methods

2.1. Plant material

The seedlings of *Solanaceae* family: rootstock cultivar Torubamu (*S. torvum*), scion cultivars Ziliren (*S. melongena*) and Aoteyou (*S. lycopersicum*), grown at the stage of 5–6 leaves, were purchased from Haicheng Sanxing Ecological Agriculture Co., LTD in Liaoning Province, China.

2.2. Grafting

The uniform seedlings above (at a growth stage of 5–6 leaves) were selected to perform grafting in early May of 2017. The method of grafting was cleft grafting from Qin et al. (2015). Seedlings were grown under the following restrictions: 20–28 °C, 200 μmol m^{-2} s^{-1} light intensity, 16 h/8 h (light/dark) period, and 60–70% relative humidity. The self-grafted and grafted seedlings were cultured for 15 d to allow the formation of the graft union. Un-grafted seedlings were used as the control. Successfully grafted plants were cultured for 15 d and then subsequently used in Cd exposures. The cultivars used in this experiment are described in further detail in Table 1.

Table 1
The cultivars of *Solanaceae* family used in experiments.

Solanaceae	Grafting treatment (cultivar)		
	Un-grafted	Self-grafted (Scion + Rootstock)	Grafted (Scion + Rootstock)
Solanum torvum	Torubamu		
Solanum melongena	Ziliren	Ziliren + Ziliren	Ziliren + Torubamu
Solanum lycopersicum	Aoteyou	Aoteyou + Aoteyou	Aoteyou + Torubamu

"+" represents grafting treatment (Scion + Rootstock).

2.3. Field experiments

2.3.1. Field site description

The experiment was carried out in field plots under natural open field conditions at the National Field Research Station of Shenyang Agro-ecosystems, which is located in south Shenyang, China (41° 31′ N, 123° 24′ E). The average annual temperature of the site is approximately 5–9 °C, and the annual precipitation in this region is 650–700 mm. The size of the plot was 3.0 m × 5.0 m. The soil at the Research Station possesses a meadow brown soil with a pH of 6.5, an organic matter content of 15.04 g kg^{-1}, and a background Cd content of 0.22 mg kg^{-1}. Additionally, the soil has a total nitrogen concentration of 0.83 g kg^{-1}, a total phosphorus concentration of 0.46 g kg^{-1}, and an available potassium concentration of 93.05 g kg^{-1}.

2.3.2. Treatments of soils and plants

Surface (0–20 cm) soil samples were removed and mixed with predetermined amounts of Cd ($CdCl_2 \cdot 2.5H_2O$ solution). Resulting soil was mixed and was left to equilibrate for one day and then added back to the area in a uniform manner. The Cd concentration was measured and was approximately 2 mg kg^{-1} in the resulting soil.

Successfully grafted seedlings were then transplanted in the field in early June of 2017. The plot was divided into 4 rows with 9 plants being used in each row; space between the plants and between the rows was 50 cm and 60 cm, respectively. Every treatment (Table 1) was carried out with six replicates. During the experiment, plants were watered using tap water (with a 40%–60% of field moisture capacity) and supplied with a basal application of fertilizer containing 20 g/m^2 N, 11 g/m^2 P, and 7 g/m^2 K in the form of compound fertilizer (Stanley ® San An 20-26-8, Stanley Agriculture Group co., LTD). No further additions of fertilizer were used during plant growth. The average temperature during the exposure time period was 20–28 °C. After 3 months, roots and leaves of cultivars were collected to analyze the concentrations of Cd, total sulfur, and SO_4^{2-} during September.

2.4. Determination of Cd, total sulfur, and SO_4^{2-} concentrations

Harvested roots and leaves were dried at 75 °C to achieve a constant weight. Dried roots and leaves were ground to a fine powder and passed through a 0.149 mm sieve. Each sample (0.2–0.5 g) was digested in a microwave with a mixture of HNO_3 and H_2O_2 (5:1, v/v) to analyze Cd and total sulfur concentrations (Arao et al., 2008). SO_4^{2-} was extracted by homogenizing the samples (0.2–0.5 g) in an ice-cold solution of 1:10 (w/v) 0.1 N HNO_3; after 10 min of heating at 100 °C, the extracts were then filtered and subsequently used for SO_4^{2-} analysis (Nocito et al., 2002). The digested samples were used to determine concentrations of Cd, total sulfur, and SO_4^{2-} by inductively coupled plasma-mass spectrometer (ICP-MS, Perkin Elmer, NexION 300 X, USA). A Certified Reference Material (CRM) of spinach plants (GBW10015) provided by the National Research Center for CRM, China, was used to monitor the recovery rates of plant samples.

2.5. Statistical analysis

Data were statistically analyzed using a one-way ANOVA based on Least Significant Difference (LSD) using the SPSS 20.0 software package. A $p < 0.05$ was deemed as the threshold for significance when comparing the experiment treatments.

3. Results

3.1. Characteristic of Cd accumulation in S. torvum, S. melongena, and S. lycopersicum

As shown in Table 2, there were no differences between Cd concentrations in roots of *S. torvum*, *S. melongena*, and *S. lycopersicum* when planted in soil with 2 mg kg^{-1} Cd; however, Cd concentrations in leaves were 0.76 mg kg^{-1}, 10.12 mg kg^{-1}, and 23.19 mg kg^{-1} in these three same cultivars, respectively. The enrichment factor (EF), which is used to evaluate the ability of a plant to accumulate heavy metals, was calculated by taking the ratio of the Cd concentration in shoots to the Cd concentration in soil (Chen et al., 2004). The EF in leaves of *S. torvum* was 0.38, whereas the EFs in *S. melongena* and *S. lycopersicum* were much higher (5.06 and 11.06), respectively. The translocation factor (TF) was also evaluated in the present study, and evaluates the capacity of a plant to transport heavy metals from the roots to shoots, and is calculated by taking the ratio of the Cd concentration in shoots to the Cd concentration in roots (Baker and Whiting, 2002). The TF in leaves of *S. torvum* was 0.13. The low EF and TF (<1) for *S. torvum* suggest that this crop is a low-Cd accumulated crop (Liu et al., 2009), and could be useful in grafting.

3.2. Effects of grafting on Cd accumulation in the shoots of S. melongena and S. lycopersicum

As discussed earlier, Cd concentrations in the leaves of *S. torvum* were much lower than those in *S. melongena* (eggplant) and *S. lycopersicum* (tomato), respectively (Fig. 1A and B). With *S. torvum* as rootstock, Cd concentrations were 1.11 mg kg^{-1} and 6.58 mg kg^{-1} in the leaves of grafted eggplant and tomato plants, and as such were significantly lower (89% and 72%, respectively) when compared to the un-grafted plants (Fig. 1A and B). There were no significant differences for Cd concentrations between rootstock *S. torvum* and the two plants (*S. melongena* and *S. lycopersicum*) tested (Table 3). This suggested that grafting on *S. torvum* could greatly reduce Cd accumulation in the shoots of eggplant and tomato, respectively. Additionally, Cd concentration was 12.11 mg kg^{-1} in the leaves of self-grafted eggplant, which was similar with that in the un-grafted plants (10.12 mg kg^{-1}); whereas Cd concentration was 29.47 mg kg^{-1} in self-grafted tomato, which was higher than that in un-grafted plants (23.19 mg kg^{-1}; Fig. 1A and B). Overall, these trends suggest that the grafting operation alone could affect Cd accumulation in tomato plants, but would not directly reduce Cd accumulation in eggplant or tomato plants.

3.3. Effects of grafting on sulfur in the shoots of S. melongena and S. lycopersicum

To explore the role of sulfur in grafting-mediated reduction of Cd accumulation in scions, we analyzed the concentrations of total sulfur and SO_4^{2-} in plants tested. The lowest total sulfur and SO_4^{2-} concentrations were in the leaves of *S. torvum*, and these concentrations were much lower than those in self-grafted and un-grafted eggplant and tomato, respectively (Fig. 2A and B). With *S. torvum* as rootstock, the concentrations of total sulfur and SO_4^{2-} in the leaves of eggplant and tomato were significantly reduced (Fig. 2A and B). Additionally, the total sulfur and SO_4^{2-} concentrations in the leaves of self-grafted plants were higher than those in un-grafted eggplant and tomato plants, respectively (Fig. 2A and B).

Similar trends occurred between Cd accumulation and sulfur concentrations in un-grafted, self-grafted, and grafted plants. Thus, we further analyzed the correlation between Cd concentrations and total

Table 2
Cd concentrations in roots and leaves of 3 cultivars grown in soil spiked with 2 mg kg^{-1} Cd.

Solanaceae	Cultivar	Root (mg kg^{-1} DW)	Leaf (mg kg^{-1} DW)	Leaf/soil	Leaf/root
S. torvum	Torubamu	6.07 ± 0.01^a	0.76 ± 0.05^a	0.38	0.13
S. melongena	Ziliren	7.65 ± 0.25^a	10.12 ± 1.06^b	5.06	1.32
S. lycopersicum	Aoteyou	6.78 ± 0.21^a	23.19 ± 1.95^c	11.60	3.42

Different letters indicate significant differences among cultivars ($p < 0.05$); data are means ± SD of six replicates from individual plants. DW: dry weight.

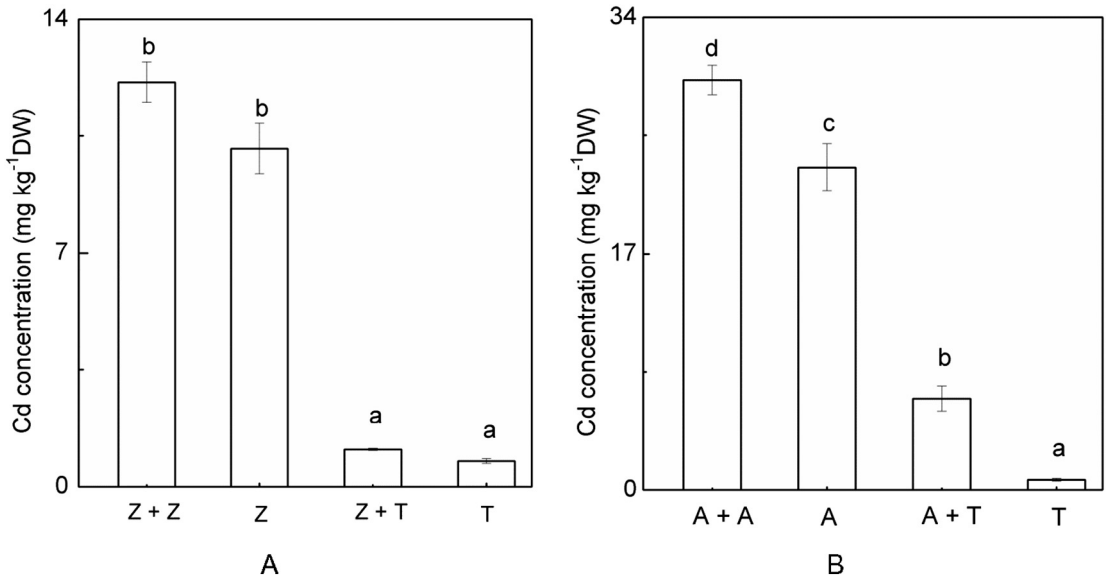

Fig. 1. Effects of grafting on Cd accumulation in leaves of *S. melongena* (A) and *S. lycopersicum* (B) grown in 2 mg kg^{-1} Cd-spiked soil. Bars and error bars are means and SD of six replicates. Different letters represent significant differences between grafting treatments ($p < 0.05$). DW, dry weight; Z: Ziliren, T: Torubamu, and A: Aoteyou. "+" represents grafting treatment (Scion + Rootstock). Z + Z: self-grafted *S. melongena*, Z: un-grafted *S. melongena*, Z + T: grafted *S. melongena* on *S. torvum* rootstock, T: un-grafted *S. torvum*, A + A: self-grafted *S. lycopersicum*, Z: un-grafted *S. lycopersicum*, Z + T: grafted *S. lycopersicum* on *S. torvum* rootstock.

sulfur/SO_4^{2-} concentrations in eggplant and tomato plants. Interestingly, we found a very strong positive correlation between Cd accumulation and total sulfur concentration in eggplant and tomato ($r^2 = 0.961$ and $r^2 = 0.986$, respectively; Fig. 3A and B); a similar strong positive correlation between Cd accumulation and SO_4^{2-} concentration was also found in eggplant, tomato ($r^2 = 0.959$ and $r^2 = 0.974$, respectively; Fig. 3A and B). Alternatively, there were no differences between total sulfur concentrations in roots of rootstock *S. torvum*, *S. melongena*, and *S. lycopersicum* (Table 3).

4. Discussion

4.1. Grafting-mediated decrease of heavy metal accumulation in the shoots of plants

It is well known that grafting can dramatically reduce heavy metal accumulation in the shoots of plants grafted on some specific rootstocks. For example, in a previous study, the concentrations of microelements B, Zn, Sr, Mn, Cu, Ti, Cr, Ni, and Cr in the fruits of grafted melon onto the commerical Cucurbita 'TZ-148' rootstock were lower than those in un-grafted plants (Edelstein and Ben-Hur, 2007). Previous studies utilizing *S. torvum* as a rootstock (as used in the present study) showed reductions of Cd of 63–74% in the fruits, stems, and leaves of *S. melongena* cultivar Senryou2 (Arao et al., 2008). Our data showed that grafting onto *S. torvum* rootstock dramatically reduced Cd concentrations by up to 89% and 72% in the leaves of eggplant and tomato, respectively (Fig. 1A and B). This finding is important at it shows that the efficiency of reducing Cd by grafting is superior to the process of regulating Cd via alterations in soil pH values, which has been shown to only reduce Cd accumulation in crops by no >50% (Arao et al., 2008). Overall, this suggests that the grafting technique could be an alternative that is an environmentally friendly strategy, which could impede Cd translocation in the shoots in agricultural cultivation of eggplant and tomato plants on *S. torvum* rootstock.

Currently, it has been speculated that both the grafting operation and the rootstock used could affect heavy metal accumulation in the shoots of plants. (i) Role of grafting operation. Qin et al. (2013) reported that Cd accumulation was decreased by 58% in the leaves of self-grafted *S. torvum* as compared to the un-grafted plants, indicating that the grafting operation could impede Cd translocation from roots to shoots. However, a previous finding showed that there was no difference for Zn/Cd accumulation between self-grafted and un-grafted *T. caerulescensa* or *T. perfoliatum* plants, suggesting that the grafting operation did not affect Zn/Cd accumulation in shoots (Guimarães et al., 2009). Similarly, in this work, Cd accumulation in the leaves of self-grafted eggplant was not significantly different from un-grafted plants (Fig. 1A). Perhaps even more interesting are reports with self-grafted soybean cultivars Suzuyutaka, Hatayutaka, and Enrei which had consistently higher shoot Cd concentrations than the un-grafted controls, suggesting that the grafting operation could actually enhance Cd accumulation in shoots of soybean (Sugiyama et al., 2007). Our findings provided similar findings as Cd accumulation was increased by 20% in self-grafted tomato as compared to the un-grafted ones (Fig. 1B). Overall, these results, suggest, at least for these two plants, that the grafting operation did not contribute to the decrease of Cd accumulation in the scions of eggplant and tomato grafted on *S. torvum* rootstock. (ii) Role of rootstock. A previous study using tomato as rootstock increased Cd concentrations in the shoots of *S. photeinocarpum* post-grafting generation compared with un-grafted seedlings (Wang et al., 2016). Obviously, this is the opposite trend as in the present study, but it shows the significance of rootstock choice, as tomato plants appears to be a rootstock that would hyper-accumulate heavy metals in scions and could enhance the ability of post-grafting generation plants to accumulate heavy metals in shoots. As such, the use of this rootstock could assist in the

Table 3
Cd and total sulfur concentrations in roots of cultivars grown in soil spiked with 2 mg kg^{-1} Cd.

Solanaceae	Grafting treatment	Cd concentration (mg kg^{-1} DW)	Total sulfur concentration (mg kg^{-1} DW)
S. torvum	Un-grafted	6.37 ± 0.25^a	2165 ± 62^a
S. melongena	Un-grafted	7.65 ± 0.01^a	2556 ± 218^a
	Self-grafted	7.21 ± 0.03^a	2506 ± 186^a
	Grafted	6.95 ± 0.05^a	2295 ± 89^a
S. lycopersicum	Un-grafted	6.78 ± 0.21^a	2248 ± 185^a
	Self-grafted	7.01 ± 0.08^a	2338 ± 74^a
	Grafted	6.58 ± 0.04^a	2236 ± 120^a

Different letters indicate significant differences among cultivars ($p < 0.05$); data are means ± SD of six replicates from individual plants. DW: dry weight. Rootstock in grafted plants was *S. torvum*.

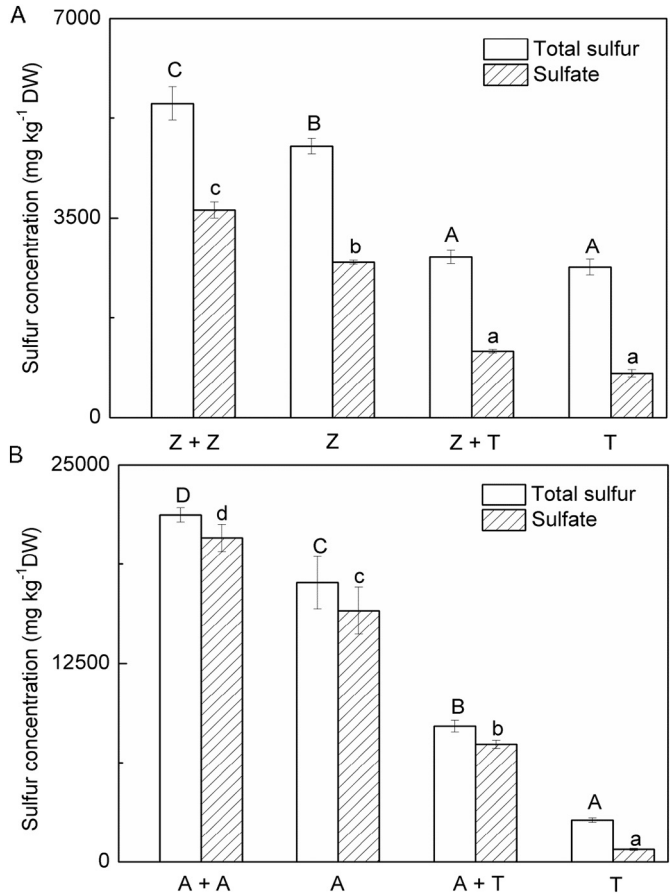

Fig. 2. Effects of grafting on sulfur concentrations in leaves of *S. melongena* (A) and *S. lycopersicum* (B) grown in 2 mg kg^{-1} Cd-spiked soil. Bars and error bars are means and SD of six replicates. Different letters represent significant differences between grafting treatments for total sulfur or sulfate, respectively ($p < 0.05$). DW, dry weight; Z: Ziliren, T: Torubamu, and A: Aoteyou. "+" represents grafting treatment (Scion + Rootstock). Z + Z: self-grafted *S. melongena*, Z: un-grafted *S. melongena*, Z + T: grafted *S. melongena* on *S. torvum* rootstock, T: un-grafted *S. torvum*, A + A: self-grafted *S. lycopersicum*, Z: un-grafted *S. lycopersicum*, Z + T: grafted *S. lycopersicum* on *S. torvum* rootstock.

remediation of contaminated heavy metal soils, showing the practical nature of rootstocks for other purposes as well. Alternatively, eggplant as rootstock decreased Cd concentrations in the shoots of *S. photeinocarpum* post-grafting generation compared with un-grafted seedlings (Wang et al., 2016). Similarly, with the commercial pumpkin 'Shintoza' as the rootstock, Cu accumulation in the leaf of grafted cucumber plants at 47 and 94 μM Cu treatment with respect to control (138% and 181%, respectively), was lower than that of un-grafted plants (about 235% and 392%, respectively; Rouphael et al., 2008). Overall, this study as well as past studies show the importance of understanding the biological characteristics of rootstocks. Additionally, it clarifies that at least in the present study, the *S. torvum* rootstock, and not the grafting operation, was responsible for the efficient reduction of Cd accumulation in the shoots of eggplant and tomato plants.

According to previous work the root capacity of the plant plays a decisive role in limiting Cd transport from roots to shoots (Sugiyama et al., 2007; Xin et al., 2013). Our results showed that Cd accumulation was not significantly different in the roots of rootstock *S. torvum* when compared to *S. melongena* or *S. lycopersicum* (Table 3), suggesting that the dramatic decreases in Cd levels in grafted eggplant and tomato plants was not attributed to Cd retention in roots, but rather the lower root-to-shoot transport of Cd in *S. torvum*. The low ability of Cd translocation in *S. torvum* plants may be related to the affinity for Cd in the xylem loading process (Mori et al., 2009; Yamaguchi et al., 2011), and also to the down-regulation of citrate transporter (*AtFRD3*) in xylem loading process (Yamaguchi et al., 2010). Additionally, observations on elemental maps of Cd accumulation in *S. torvum* and *S. melongena* root sections by synchrotron micro X-ray fluorescence spectrometry showed that Cd was mostly accumulated in the endodermis and pericycle of *S. torvum*, but was present in the stele and epidermis of *S. melongena*, demonstrating that cortex casparian strip structure may be a key barrier to impede Cd translocation from roots to shoots of *S. torvum* (Yamaguchi et al., 2011).

Rootstock could also affect gene expression in scions, as some signals transported from the root to the shoot could influence the Cd accumulation in scions (Si et al., 2010). A recent study reported an exchange of genetic information occurring in graft sites, through either large DNA pieces or entire plastid genomes, possibly resulting in a novel combination of genetic material via the grafting technique (Stegemann and Bock, 2009). In this study, the cause of the decreased Cd accumulation in grafted eggplant and tomato on *S. torvum* rootstock may be due to a specific genetic substance associated with low-Cd accumulation in *S. torvum*, which was ultimately transferred into the scions. It should be noted, however, that with *S. torvum* as rootstock, Cd concentration in the leaves of grafted eggplant was similar to that in *S. torvum* (Fig. 1A), whereas Cd in grafted tomato was much higher than that in *S. torvum* (Fig. 1B). In other words, it appears that inter-specific differences exist between eggplant and tomato plants for Cd accumulation

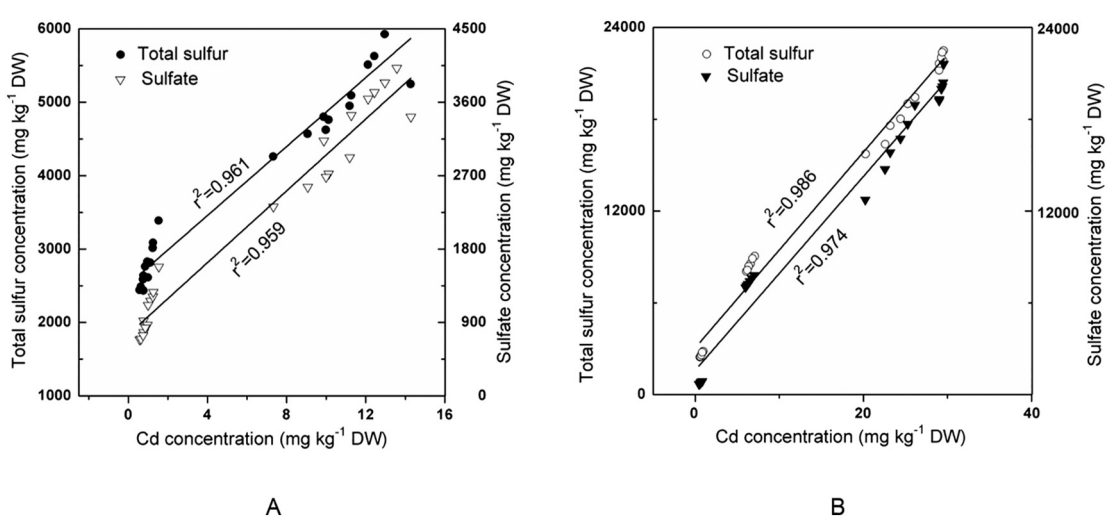

Fig. 3. Correlation between sulfur content (total sulfur, sulfate) and Cd accumulation in leaves of *S. melongena* (A) and *S. lycopersicum* (B) grown in 2 mg kg^{-1} Cd-spiked soil.

even when the same rootstock is used. One possible explanation for these differences is the ability of graft fusion was ultimately just weaker between the tomato and *S. torvum* when compared to eggplant and the rootstock, making directional transfer of genetic materials less effective.

4.2. Role of sulfur in Cd accumulation of plants

Sulfur (S) is an essential element in vegetative growth and metabolism, and it may be involved directly and/or indirectly in heavy metal accumulation of plants (Zhang et al., 2012). Our results revealed that there was a similar trend between sulfur concentrations and Cd concentrations in the leaves of tested plants (Figs. 1A–B and 2A–B). Similarly, X-ray fluorescence (XRF) observations in previous work showed a final spatial distribution pattern of S similar to that of Cd in rice seedlings, and that the S supply facilitated Cd translocation from roots to the top of shoots (Zhang et al., 2013). In fact, many studies have showed that S-containing compounds may be an underlying factor in Cd accumulation of plants (Liang et al., 2014; Zhang et al., 2012). In the present study, a strong positive correlation was found between Cd accumulation and sulfur concentration (total sulfur or SO_4^{2-}) in leaves (Fig. 3A–B), whereas total sulfur concentrations in roots of rootstock *S. torvum*, *S. melongena*, and *S. lycopersicum* were not significantly different (Table 3). Root as a tissue organ is mainly used to take in water and minerals from soil, but also serves as a conduit to absorb various trace elements such as Ni, Cu, Pb, Mg, Mn, Zn, and Cd. The absorption process for this tissue is easily affected by a variety of factors in the environment (such as soil pH, Eh, etc.), and thus sulfur concentration alone in roots cannot fully explain Cd accumulation in shoots. Whereas leaf as an organ, is the location for the synthesis of organic matter, such as proteins, and as the ultimate destination for many trace elements, it is a single and special environment, hence sulfur in shoots with a strong correlation with Cd concentrations may be particularly responsible for the accumulation of Cd in leaves. Therefore, S, mainly SO_4^{2-} in leaves, may be a potential factor in affecting Cd accumulation in crops. It is likely that S-containing compounds may have some particular structure (such as disulfide bond or —SH) to chelate Cd. For example, PCs have been regarded as transporters in long-distance Cd translocation from roots to shoots in other studies (Chen et al., 2006; Gong et al., 2003), while others have shown other compounds, such as nicotianamine (NA) may play an important role in Cd hyperaccumulation and detoxification (via a study with *Sedum alfredii* Hance under low Cd levels) (Liang et al., 2014). NA in particular, is synthesized from *S*-adenosylmethionine (SAM) catalyzed by nicotianamine synthase (NAS), and SAM is produced from Met, an S-containing amino acid. Thus, it is possible that different S-containing compounds were used to synthesize different Met, a precursor of NA, to cope with Cd stress caused by different Cd accumulation in plants.

On the other hand, S-containing compounds also played an important role in mitigating Cd stress, wherein PCs are regarded as a major detoxification mechanism by compartmentalization of PC-Cd complexes in vacuoles (Chen and Guo, 2014; Cobbett, 2000; Fan et al., 2010; Liang et al., 2016). In response to heavy metals, plants activate the sulfur assimilation pathway to up-regulate S assimilation by raising the activity of some relevant enzymes (such as ATP sulfurylase, APS reductase, sulfite reductase, and *o*-acetylserine (thiol) lyase) providing an increasing GSH supply for biosynthesis of PCs (Harada et al., 2002; Liang et al., 2014; Liang et al., 2016). In terms of physiology, plants need to synthesize some metabolites, such as GSH and cysteine, to resist Cd stress by increasing SO_4^{2-} absorption and reduction. For example, Cd accumulation induced SO_4^{2-} uptake by increasing the levels of mRNA encoding a putative high-affinity sulfate transporter in roots (Nocito et al., 2002). Hence, SO_4^{2-} accumulation in leaves may be attributed to the induction of Cd accumulation in plants. However, specific S-containing compounds responsible for Cd accumulation in plants need to be explored in future studies to better understand this phenomenon.

5. Conclusion

This study demonstrated that grafting techniques could be a useful tool to reduce Cd accumulation in leaves of eggplant and tomato with *S. torvum* as rootstock. The present study revealed that it was the *S. torvum* rootstock that was responsible for the decreased Cd accumulation in scion plants. In addition, there was a strong positive correlation between Cd accumulation and total sulfur or SO_4^{2-} concentrations in leaves, suggesting that S, mainly SO_4^{2-}, in leaves may play an important role in Cd accumulation in plants tested. Overall, the results of the present study show that *S. torvum* would be a good rootstock at the very least for tomato and eggplant in soils with substantial Cd loadings, although further work is needed with other crops, the potential is present for other major crops as well.

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