

Cadmium stress in Dongying wild soybean seedlings: growth, Cd accumulation, and photosynthesis

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Abstract

In order to understand better Cd resistance in soybean, Dongying wild soybean treated with different Cd concentrations were evaluated. The biomass, chlorophyll (Chl) content, leaf color, Chl *a* fluorescence parameters, photosynthesis parameters, and Cd contents were determined. Our results showed that when Cd concentration was $\leq 2 \text{ kg m}^{-3}$, no significant decrease in biomass, photosynthetic parameters, and maximal photochemical efficiency of PSII was observed. This indicated that Dongying wild soybean resisted Cd toxic effects under such conditions. In addition, atomic absorption experiment results demonstrated that when Cd concentration was $\leq 0.5 \text{ kg m}^{-3}$, the accumulation of Cd in wild soybean was lower in roots than that in shoots, while the accumulation of Cd was higher in roots than that in shoots when Cd concentration was $\geq 1 \text{ kg m}^{-3}$. Therefore, Dongying wild soybean showed a certain resistance to Cd and could serve as a valuable germplasm resource for improving the breeding of Cd-resistant soybean.

Additional key words: atomic absorption spectrophotometry; *Glycine soja*; growth analysis; pigment.

Introduction

Heavy metal Cd is widely spread in the nature and it is a non-nutritive element in plants. Cd content is generally around 0.01–0.7 mg kg⁻¹ in soil (Kovalchuk *et al.* 2001, Ūnyayar *et al.* 2006). The Cd content in cultivated lands has increased from the use of chemical substances and mine exploitation that go hand-in-hand with city development. In recent years, the metal pollution of farm land affected about 20% of the arable land in China, and the pollution by Cd was found to be higher than those of other heavy metals (Wei *et al.* 2010, Wang *et al.* 2015). Cd in soil has become a widespread phenomenon in China, threatening the health of people (Zhao *et al.* 2010). The second large-scale survey in China showed that the standard rate of Cd pollution was 7% among all sampling sites (Wang *et al.* 2015). Cd in rural soil was 1.121 mg kg⁻¹, while that in urban soils it was 0.357 mg kg⁻¹ in Sichuan (Wang *et al.* 2015).

Due to its high toxicity and solubility, Cd is a particularly dangerous pollutant (Duxbury *et al.* 1985, Jiang *et al.* 2001). The growth of plants is extremely

inhibited by Cd stress. For example, the root development and cell division in *Allium cepa*, *Tradescantia ohiensis*, *Vicia faba*, *Nicotiana tabacum*, and *Allium sativum* have been reported to be inhibited by Cd (Steinkellner *et al.* 1998, Fojtová *et al.* 2000, Yi *et al.* 2003). Moreover, the physiological and biochemical processes of plants, such as photosynthetic reactions (Wu *et al.* 2007, Sun *et al.* 2008, Ci *et al.* 2010) and Chl biosynthesis (Myśliwa-Kurdziel *et al.* 2002), have been also shown as affected. When exposed to Cd, Lhcb1 and Lhcb2 were observed to be dissipated and LHCII aggregation complexes were found to be diminished in plants (Janik *et al.* 2010). In addition, both acceptor and donor sides of PSII were affected which led to the prevention of photoactivation (Sigfridsson *et al.* 2004). In addition, PSI was found to be damaged under Cd stress (Timperio *et al.* 2007). Taken together, photosynthesis is extremely inhibited by Cd stress.

The subgenus *soja* in *Glycine* includes only two species: the cultivated soybean [*Glycine max* (L.) Merr.] and wild soybean (*Glycine soja* Sieb. et Zucc.)

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Abbreviations: Car – carotenoids; Chl – chlorophyll; CF – chlorophyll fluorescence; C_i – intercellular CO₂ concentration; DM – dry mass; E – transpiration rate; FM – fresh mass; F₀ – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v – variable fluorescence; F_v/F_m – maximal photochemical efficiency of PSII; g_s – stomatal conductance; L_s – stomatal limitation; MC – mesophyll conductance; P_N – net photosynthetic rate; WUE – water-use efficiency (= P_N/E).

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(Wang *et al.* 2011). The cultivated soybean, as the main food crop in the world, has been reported to be Cd sensitive (Finger-Teixeira *et al.* 2010). It is hypothesized that the yield of cultivated soybean could increase by enhancing its tolerance to Cd. However, the limited germplasm resources and genetic knowledge on cultivated soybean have become bottlenecks for soybean breeding (He *et al.* 2016). Wild soybean, the direct precursor of cultivated soybean, widely populates the East Asian continent and the Japanese archipelago (He *et al.* 2016). A wide spectrum of ecological and morphological types of Chinese wild soybean has been observed (Wang *et al.* 2001), which provides an original source of information on genes and traits for cultivating and breeding soybeans (Wang *et al.* 2011). The genetic base of wild soybean demonstrates biological diversity and evidence of eminent resistance genes and serves as an important germplasm resource for genetic improvement of cultivated soybean.

G. soja is widely distributed in Dongying of Shangdong province, which belongs to the Yellow River

Materials and methods

Cultivation conditions: Several hundred Dongying wild soybean seeds were scratched with sandpapers and disinfected with 0.1% HgCl₂ solution for 10 min, and then washed with deionized water about ten times. The seeds were then kept in the deionized water at room temperature overnight. On the next day, the seeds were put in large standard Petri dishes (50 seeds per each dish), and incubated in a growth chamber (28/22°C, 16/8 h, 80% humidity) until the first cotyledons appeared. After 7 d, the healthy seedlings that were growing consistently were transplanted into polyethylene pots (diameter of 18 cm, depth of 20 cm) with vermiculite and perlite (3:1, v/v) mixed with 1/2 Hoagland solutions. A total of eight seedlings were placed in each pot. Seven days later, five consistent seedlings from each pot were kept.

Solutions containing different Cd concentrations (0, 0.25, 0.5, 1, 2, 4, and 8 kg m⁻³) were prepared with CdCl₂·2.5H₂O. Three pots containing 15 seedlings were used in each treatment. Throughout the entire cultivation period, the seedlings were treated with 500 ml of each solution once a week.

Leaf color was observed at 30 d after exposure to Cd stress. The biomass, Chl and carotenoids (Car) content, photosynthetic traits, and atomic absorption parameters were measured at 60 d after stress.

Leaf color and biomass: Pictures of the secondary leaves from the parietal lobes were taken under a stereomicroscope (*Olympus SZX16*, *Olympus*, Tokyo, Japan). For biomass, ten plants were selected randomly from each treatment. The shoots and the roots of seedlings were washed with distilled water, then placed in an oven (70°C) overnight until their masses were constant.

delta in China. This area is known for its saline and alkaline content and plants in Dongying generally demonstrated a certain resistance to salt and alkali (Wang *et al.* 2016). Previous report has demonstrated that Mediterranean saltbush, *Atriplex halimus* L., is capable of growing in saline alkali soil and is Cd tolerant (Manousaki *et al.* 2009). Based on this observation, we infer that Dongying wild soybean may also harbor certain tolerance to Cd. If this ecotype of wild soybean is resistant to Cd, it could serve as an important germplasm resource for breeding Cd-resistant cultivated soybean.

In the present study, Dongying wild soybean was treated with solutions of different Cd concentrations (0–8 kg m⁻³). The biomass, photosynthetic pigment content, chlorophyll fluorescence (CF) parameters, photosynthesis traits, and the Cd contents were investigated. Furthermore, Cd tolerance of Dongying wild soybean was also assessed. Our results could provide important scientific insights for future studies on breeding Cd-resistant cultivated soybean.

Chl and Car contents: Ten plants at the same developmental stage were selected from each treatment group and the secondary leaves from the parietal lobes were harvested. Chl and Car were isolated according to Li *et al.* (2013). The Chl and Car contents were measured by UV-VIS spectrophotometer (*UV-1750*, *Shimadzu*, Kyoto, Japan) (Cherian *et al.* 2008).

Chl fluorescence was measured in intact leaves with a multifunctional modulation fluorescence imaging system (*MINI-IMAGING-PAM*, *Walz*, Effeltrich, Germany). The leaves were kept in darkness for 30 min. The minimal fluorescence yield of the dark-adapted state (F_0), the maximal fluorescence yield of the dark-adapted state (F_m), and variable fluorescence (F_v) were measured. F_0 induced by weak modulation light [$0.05 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] were measured using the back of the leaf. F_m was induced by strong saturation flash [$6,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$]. Ten readings were collected for each treatment. F_v/F_m was calculated using the formula $F_v = F_m - F_0$ (Küpper 2007, Li *et al.* 2013, Khan *et al.* 2015).

Photosynthetic traits: Characteristics were assessed using portable photosynthesis instrument (*GFS-3000*, *Walz*, Effeltrich, Germany). The net photosynthetic rate (P_N) was measured at 14:00–16:00 h. Stomatal conductance (g_s), intercellular CO₂ concentration (C_i), and transpiration rate (E) were measured using the secondary leaves from the phyllophore. The temperature, relative humidity, and ambient CO₂ concentration at the time of measurement were $31.1 \pm 0.9^\circ\text{C}$, $62.6 \pm 2.0\%$, and $3.3 \pm 0.1 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$, respectively. Water-use efficiency (WUE), mesophyll conductance (MC), and stomatal limitation (L_s)

were calculated according to Wei *et al.* (2004), Li *et al.* (2013), and Nwugo *et al.* (2008).

Cd contents: The experiments were pretreated according to Nwugo *et al.* (2008). The Cd contents of different tissues were measured with a continuous light source atomic absorption spectrophotometer (*ContraAA700, Analytik Jena, Jena, Germany*).

Results

Biomass: Comparing with control, the DM of shoots, roots, and whole plants decreased when the concentration of Cd increased to 2 kg m⁻³, however, no significant difference was observed (Table 1). Interestingly, a significant downward trend was observed regarding the DM of shoots, roots, and whole plants when Cd concentrations were further increased to 4 kg m⁻³ and 8 kg m⁻³ (Table 1).

Leaf color: The leaf color of the control started as green (Fig. 1A), which gradually faded with increasing Cd concentration. When the Cd stress concentration reached 2 kg m⁻³, the leaf color was Kelly green (Fig. 1B). When the Cd concentration was at 8 kg m⁻³, lots of maculopathy were observed on the now yellow leaves (Fig. 1C).

Table 1. Effects on the biomass of wild soybean upon exposure to Cd stress. Values represent mean ± SD. a, b – the significant differences at $p = 0.05$. DM – dry mass.

Cd [kg m ⁻³]	Shoot DM [g]	Root DM [g]	Total plant DM [g]
0.00	1.12 ± 0.18 ^a	0.80 ± 0.02 ^a	1.92 ± 0.20 ^a
0.25	0.77 ± 0.01 ^{ab}	0.58 ± 0.10 ^a	1.36 ± 0.11 ^{ab}
0.50	0.67 ± 0.15 ^{ab}	0.48 ± 0.43 ^a	1.15 ± 0.58 ^{ab}
1.00	0.86 ± 0.50 ^a	0.39 ± 0.22 ^a	1.24 ± 0.72 ^a
2.00	0.87 ± 0.39 ^a	0.52 ± 0.29 ^a	1.39 ± 0.68 ^a
4.00	0.44 ± 0.10 ^b	0.17 ± 0.03 ^b	0.61 ± 0.06 ^b
8.00	0.30 ± 0.17 ^b	0.10 ± 0.07 ^b	0.41 ± 0.24 ^b

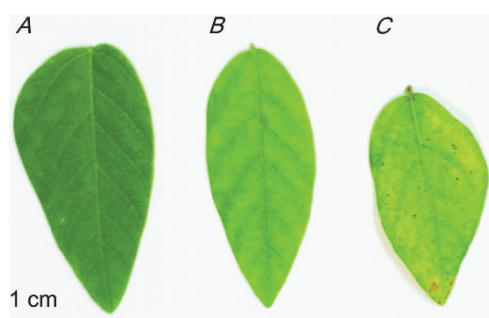


Fig. 1. The leaf color of Dongying wild soybean. Photos were taken after 30-d exposure to Cd stress; A, B, C: Cd concentrations of the treatment solution were 0, 2, and 8 kg m⁻³, respectively.

Statistical analyses: All experiments were repeated three times. The values in tables represent means ± SD. Statistical analyses were performed with *Microsoft Excel* and analyses of variance (*ANOVA*) by *SPSS (ver. 17 Inc.)*. Statistical significance was assessed by *Duncan's* multiple range test ($p < 0.05$). The graphs were drawn using *Origin 8.1*.

Chl and Car: As Cd concentration increased to 0.5 kg m⁻³, the Chl *b* and Car contents also increased significantly compared with control. Interestingly, as the Cd concentrations further increased to 8 kg m⁻³, Chl and Car contents gradually decreased and a significant reduction was observed in comparison with the control (Table 2).

Table 2. The concentrations of Chl in wild soybean under Cd stress. Values represent mean ± SD. a, b, c – the significant differences at $p = 0.05$. Chl – chlorophyll; Car – carotenoids; FM – fresh mass.

Cd [kg m ⁻³]	Chl <i>a</i> [mg g ⁻¹ (FM)]	Chl <i>b</i> [mg g ⁻¹ (FM)]	Car [mg g ⁻¹ (FM)]
0.00	7.171 ± 0.546 ^c	1.740 ± 0.133 ^{cd}	318.553 ± 23.334 ^{cd}
0.25	7.946 ± 0.311 ^c	2.148 ± 0.184 ^{de}	376.605 ± 18.873 ^d
0.50	8.307 ± 0.746 ^c	2.302 ± 0.212 ^e	454.955 ± 16.062 ^e
1.00	7.106 ± 0.355 ^c	1.843 ± 0.092 ^{cd}	328.039 ± 16.402 ^{cd}
2.00	5.636 ± 0.548 ^b	1.468 ± 0.113 ^{bc}	302.370 ± 32.918 ^{bc}
4.00	4.590 ± 0.186 ^{ab}	1.164 ± 0.055 ^{ab}	244.980 ± 2.370 ^{ab}
8.00	3.999 ± 0.210 ^a	0.992 ± 0.045 ^a	204.223 ± 3.097 ^a

Chl fluorescence: F_0 significantly increased by 8.6% compared with the control when the Cd concentration was 0.25 kg m⁻³. As the Cd concentration reached 4 kg m⁻³, no significant difference was observed compared with control. Interestingly, when the Cd concentration reached 8 kg m⁻³, F_0 significantly increased by 14.2% compared with control (Fig. 2A).

When Cd concentration was ≤ 0.5 kg m⁻³, no significant difference in F_m was observed between treatment groups and control. With increasing Cd concentrations, however, F_m significantly decreased by 3.4, 5.1, 6.5, and 2.7% compared with control, respectively (Fig. 2B).

With increasing Cd concentration, F_v/F_m showed an obvious downward trend (Fig. 2C). F_v/F_m was significantly reduced by 11.5, 2.8, 3.7, 7.2, 9.0, and 13.2%, respectively, under increasing Cd concentration treatments. It is worth noting that F_v/F_m significantly decreased at 0.25 kg m⁻³ Cd. Interestingly, no significant difference from the group treated with 0.25 kg m⁻³ Cd was observed when Cd concentration increased to 8 kg m⁻³ (Fig. 2C).

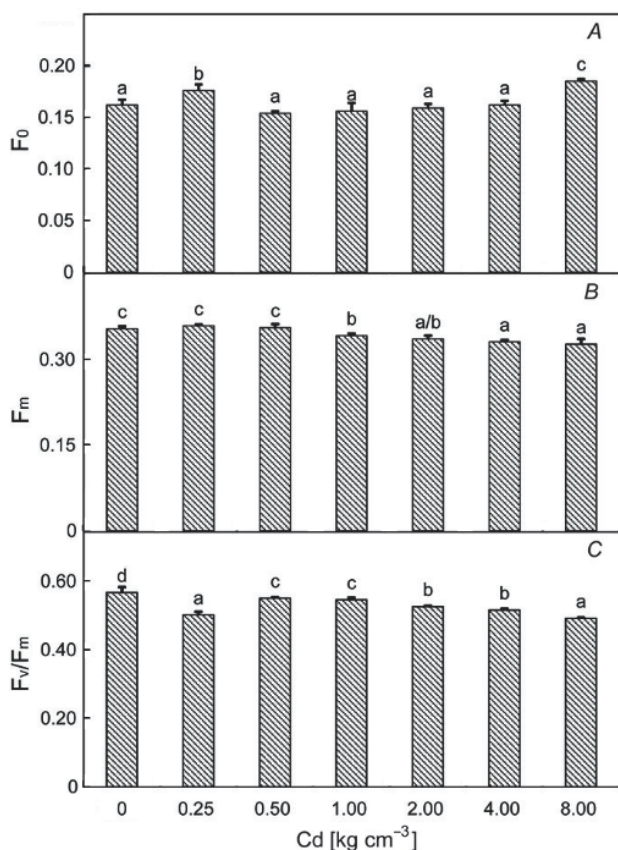


Fig. 2. Chlorophyll fluorescence parameters of Dongying wild soybean upon exposure to Cd stress. F_0 induced by weak modulation light [$0.05 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] was measured on the abaxial leaf surface. F_m induced by strong saturation flash [$6,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$]. Ten readings were collected for each treatment. The experiments were repeated three times. F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v/F_m – maximal photochemical efficiency of PSII.

Photosynthetic characteristics: No significant changes were observed in P_N , WUE, C_i , L_s , and MC with increasing Cd concentrations. However, g_s significantly increased when Cd concentrations were 0.25 and 8 kg m^{-3} . E also significantly increased when the plants were treated with 0.25 and 8 kg m^{-3} Cd (Table 3).

Cd content in different tissues: The regression coefficient and the linear regression of the Cd standard curve were $y = 0.1745x + 0.0012$ and $R^2 = 0.9843$, both were valid for calculating the Cd contents (Fig. 3). The distributions of Cd in seedlings exhibited a certain regularity. As shown in Table 4, with the increasing Cd concentration, the Cd contents in different tissues also significantly increased. When Cd concentration was $\leq 0.5 \text{ kg m}^{-3}$, the Cd content in roots was always lesser than that in shoots. When Cd concentration was $\geq 1 \text{ kg m}^{-3}$, the Cd content in roots was always greater than that in shoots. In the control, the Cd content in stems was higher than that in leaves. However, in the treatment groups, the Cd content in stems was lesser than that in leaves.

Discussion

Cd directly or indirectly inhibits a variety of physiological processes, such as respiration, photosynthesis, water-related processes, and gas exchange (van Assche and Clijsters 1990, Lagriffoul *et al.* 1998). Under Cd stress, Chl content and F_v/F_m decreased, which indicates impeded photosynthesis (Parmar *et al.* 2013). In this study, with increasing Cd concentrations, leaf color was found to become more yellow, which implied that Chl pigment synthesis in wild soybean was inhibited. This observation

was consistent with the results shown in Table 2, which demonstrated that the Chl contents were reduced significantly upon exposure to Cd stress. Decreasing Chl content in leaves also limits photosynthesis. On the other hand, F_v/F_m in seedlings was significantly reduced, which suggested lower functional activity of PSII (Stoeva and Bineva 2003) (Fig. 2C). Photosynthesis inhibition may be attributed to diminished Chl biosynthesis (Shukla *et al.* 2008).

Table 3. Effects on photosynthetic parameters of wild soybean upon exposure to Cd stress. Values represent mean \pm SD. a, b – the significant differences at $p = 0.05$. P_N – net photosynthetic rate; g_s – stomatal conductance; E – transpiration rate; WUE – water-use efficiency ($= P_N/E$); C_i – intercellular CO_2 concentration; L_s – stomatal limitation; MC – mesophyll conductance.

Cd [kg m^{-3}]	P_N [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	g_s [$\text{mmol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$]	E [$\text{mmol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$]	WUE [$\mu\text{mol}(\text{CO}_2) \text{mmol}^{-1}(\text{H}_2\text{O})$]	C_i [$\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$]	L_s [%]	MC [$\text{mmol m}^{-2} \text{s}^{-1}$]
0.00	2.504 ± 0.473^a	28.750 ± 2.069^a	0.58 ± 0.02^a	4.317 ^a	320.927 ± 15.173^a	30.99 ^a	0.008 ^a
0.25	3.122 ± 0.035^a	65.586 ± 8.797^b	1.17 ± 0.35^b	2.665 ^a	319.732 ± 46.098^a	25.73 ^a	0.010 ^a
0.50	1.888 ± 1.205^a	32.882 ± 5.874^a	0.54 ± 0.09^a	0.749 ^a	354.776 ± 42.284^a	20.77 ^a	0.001 ^a
1.00	2.272 ± 0.014^a	37.179 ± 4.348^a	0.73 ± 0.12^{ab}	3.119 ^a	336.638 ± 5.566^a	24.89 ^a	0.007 ^a
2.00	2.344 ± 1.138^a	30.812 ± 7.205^a	0.62 ± 0.16^a	3.751 ^a	334.664 ± 28.854^a	26.96 ^a	0.007 ^a
4.00	1.570 ± 0.023^a	19.114 ± 1.447^a	0.33 ± 0.03^a	4.769 ^a	301.314 ± 12.244^a	32.46 ^a	0.005 ^a
8.00	2.235 ± 1.756^a	58.687 ± 1.925^b	1.24 ± 0.05^b	1.801 ^a	379.659 ± 42.768^a	15.73 ^a	0.006 ^a

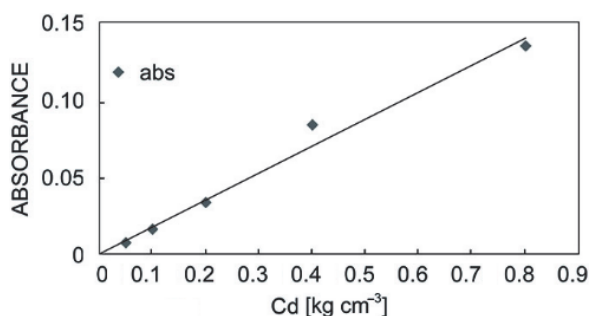


Fig. 3. Standard curve of solution at different Cd concentrations. The regression coefficient and the linear regression of the Cd standard curve were $y = 0.1745x + 0.0012$ and $R^2 = 0.9843$.

Excess Cd in crops can disturb a series of physiological processes including respiration, photosynthesis, and nitrogen assimilation, resulting in growth inhibition (Toppi and

Gabrielli 1999). The fact that growth of wildtype soybean was not affected by $\leq 2 \text{ kg(Cd}^{2+}) \text{ m}^{-3}$ is consistent with previously published results (Stritsis and Claassen 2013, Cornu *et al.* 2016). Chl biosynthesis is a physiological phenomenon linked to photosynthetic productivity of plants. Cd has been reported to inhibit Chl content in several plant systems (Vassilev *et al.* 1998, Jain *et al.* 2007). Results from this study demonstrated that Chl contents slightly increased at $\leq 0.5 \text{ kg(Cd) m}^{-3}$. Moreover, when treated with $0.25 \text{ kg(Cd) m}^{-3}$, F_v/F_m was significantly reduced. However, when Cd concentration increased to 4 kg m^{-3} , F_v/F_m increased significantly compared to that treated with $0.25 \text{ kg(Cd) m}^{-3}$ (Fig. 2). Low dose stimulation and high dose inhibition by Cd, termed as 'hormesis', has been observed in several organisms (Kumar *et al.* 2004, Jain *et al.* 2007, Cornu *et al.* 2016). For g_s , there was no significant difference when the Cd concentration was $\leq 4 \text{ kg m}^{-3}$ except for $0.25 \text{ kg(Cd) m}^{-3}$ (Table 3).

Table 4. The Cd content in different tissues of wild soybean under Cd stress. Values represent mean \pm SD. a, b, c, d, e, f – the significant differences at $p = 0.05$. Cd – cadmium; DM – dry mass.

Cd [kg m^{-3}]	Cd contents in different tissues of wild soybean [$\text{mg g}^{-1}(\text{DM})$]				
	Root	Steam	Leaf	Shoot	Plant
0.00	0.0187 ± 0.0065^a	0.0144 ± 0.0048^a	0.0110 ± 0.0090^a	0.0254 ± 0.0138^a	0.0440 ± 0.0073^a
0.25	0.0690 ± 0.0071^a	0.0209 ± 0.0082^{ab}	0.0527 ± 0.0021^{ab}	0.0736 ± 0.0102^b	0.1425 ± 0.0173^{ab}
0.50	0.1010 ± 0.0115^a	0.0380 ± 0.0016^{abc}	0.0852 ± 0.0146^{bc}	0.1232 ± 0.0162^c	0.2242 ± 0.0048^b
1.00	0.3853 ± 0.0593^b	0.0448 ± 0.0044^{bc}	0.1236 ± 0.0023^{cd}	0.1684 ± 0.0021^{cd}	0.5537 ± 0.0572^c
2.00	0.4569 ± 0.0164^b	0.0541 ± 0.0051^c	0.1470 ± 0.0021^d	0.2010 ± 0.0071^d	0.6579 ± 0.0092^c
4.00	0.8202 ± 0.0492^c	0.0559 ± 0.0050^c	0.2220 ± 0.0301^e	0.2779 ± 0.0252^e	1.0980 ± 0.0240^d
8.00	3.4427 ± 0.1264^d	0.0915 ± 0.0155^d	0.4181 ± 0.0026^f	0.5096 ± 0.0129^f	3.9522 ± 0.1135^e

Cd that entered the guard cells and combined with Ca^{2+} causes stomatal closure, which leads to a decrease in CO_2 uptake or utilization. This ultimately results in overall inhibition of photosynthesis (Perfus-Barbeoch *et al.* 2002, Pietrini *et al.* 2010). In this study, the conductance of CO_2 was not significantly affected when Cd concentration was $\leq 4 \text{ kg m}^{-3}$ (Table 3). Taken together, Dongying wild soybean responded to the abiotic stimuli and adapted to the environment when Cd concentrations was $\leq 2 \text{ kg m}^{-3}$.

Plants can reduce Cd toxicity through a variety of mechanisms, including excretion and the production of ROS scavengers and Cd-binding factors (Siripornadulsil *et al.* 2002). The ability of plants to accumulate different amounts of Cd is tissue-specific, and plays a predominant role in the adaptation to Cd stress (Wang *et al.* 2008). In shoots, the allocation of Cd to old leaves appears to be enhanced under Cd stress (Maria *et al.* 2013). In roots, the restricted movement of Cd was observed both in the symplast and the apoplast, which can be attributed to the sequestration of Cd in vacuoles and to the development of

apoplastic barriers, respectively. This phenomenon is referred to as the sequestration of Cd in plant roots (Lux *et al.* 2011). In this study, the distribution of Cd in seedlings was lesser in root than that in shoots at low Cd concentration ($\leq 0.5 \text{ kg m}^{-3}$); while more distribution of Cd was observed in roots than that in shoots at the high Cd concentration ($\geq 1 \text{ kg m}^{-3}$) (Table 4). These results indicated that Cd crosses the plasma membrane *via* Ca^{2+} channels into the cytoplasm as suggested in Li *et al.* (2017).

Conclusion: Our results indicated that Dongying wild soybean seemed to show stronger tolerance to Cd at low Cd^{2+} concentration than at high Cd^{2+} concentration. It is supported by the mild promotion of dry mass, Chl content, and photosynthetic characteristics in Dongying wild soybean at lower Cd^{2+} concentration. Therefore, we proposed that Dongying wild soybean could serve as a valuable germplasm resource for improving the breeding of Cd tolerant soybean.

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