Submitted to ***Journal of Hazardous Materials***

Are males and females of Populus cathayana differentially sensitive to Cd stress?

Miao Liu [1], Xingxing Liu [2], Jieyu Kang [1],

Helena Korpelainen [3] and Chunyang Li [1,*]

[1] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China

[2] State Key Laboratory of Plant Physiology and Biochemistry, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China

[3] Department of Agricultural Sciences, Viikki Plant Science Centre, University of Helsinki, P.O. Box 27, FI-00014, Finland

* Corresponding author: Chunyang Li, E-mail address: licy@hznu.edu.cn

Head title: Sexually different responses to Cd in poplar

Abstract This study clarifies the mechanisms of Cd uptake, translocation and detoxification in *Populus cathayana* Rehder females and males, and reveals a novel strategy for dioecious plants to cope with Cd contamination. Females exhibited a high degree of Cd uptake and root-to-shoot translocation, while males showed extensive Cd accumulation in roots, elevated antioxidative capacity, and effective cellular and bark Cd sequestration. Our study also found that Cd is largely located in epidermal and cortical tissues of male roots and leaves, while in females, more Cd was present in vascular tissues of roots and leaves, as well as in leaf mesophyll. In addition, the distributions of sulphur (S) and phosphorus (P) were very similar as that of Cd in males, but the associations were weak in females. Scanning electron microscopy and energy spectroscopy analysess suggested that the amounts of tissue Cd were positively correlated with P and S amounts in males, but not in females (a weak correlation between S and Cd). Transcriptional data suggested that Cd stress promoted the upregulation of genes related to Cd uptake and translocation in females, and that of genes related to cell wall biosynthesis, metal tolerance and secondary metabolism in males. Our results indicated that coordinated physiological, microstructural and transcriptional responses to Cd stress endowed superior Cd tolerance in males compared with females, and provided new insights into mechanisms underlying sexually differential responses to Cd stress.

Keywords**:** dioecy; sexual differences; Cd stress; Cd uptake; Cd sequestration; transcripts.

1. Introduction

Cadmium (Cd) is a non-essential and highly toxic heavy metal for plants, animals and humans. The Cd contamination of soil has become an increasingly serious problem because of the rapid industrial development and the release of agrochemicals into the soil. Therefore, it is critically important to develop means for the remediation of Cd contaminated soil. Phytoremediation is regarded as an effective and environmentally friendly technology to reduce or remove toxic heavy metals, such as Cd, from the soil (Zhao and Huang, 2018). Understanding the physiological and molecular mechanisms of plants' responses to Cd stress is vitally important when developing more effective phytoremediation methods.

Plants have evolved a series of adaptive strategies to reduce Cd damage. Cell walls are the first barrier for ion flux into the cell cytosol. Cd is largely accumulated in cell walls through binding to the uronic acids of pectin and hemicelluloses (Meyer et al., 2015; Peng et al., 2017). The upregulation of the Arabidopsis *PLANT DEFENSIN 2 gene AtPDF2.5* has been shown to cause Cd accumulation in cell walls and the enhancement of Cd tolerance (Luo et al., 2019). In addition, Cd storage in vacuoles has been reported to play a vital role in heavy metal sequestration and detoxification (Sharma et al., 2016; Zhang et al., 2018). Cd can be chelated by phytochelatins, glutathione and metallothioneins, and then transported and sequestrated into vacuoles (Hasan et al., 2015). The elevated expression of genes related to heavy metal transporters in tonoplasts in the plant shoots has been proposed to facilitate Cd detoxification in plants (Wong et al., 2009; Liu et al., 2017). Also, Cd stimulates oxidative activities of triphosphopyridine nucleotides and causes a reactive oxygen species (ROS) burst, which is detrimental to plant cells (Gupta et al., 2017). To alleviate the damage of ROS to cells, plants initiate antioxidant systems to scavenge them.

In poplar, willow and *Morus alba* L., Cd sequestrations into cell walls, vacuoles and golgi apparatus have been found to facilitate Cd detoxification (Vollenweider et al., 2006; He et al., 2013; Huang et al., 2018; Bi et al., 2020). It has been reported that Cd could be chelated by S-containing compounds, such as glutathione (GSH) and phytochelatin, and then transported into vacuoles (He et al., 2015). The overexpression of bacterial γ-glutamylcysteine synthetase facilitates Cd detoxification in the cytosol of *Populus tremula* × *P. alba* (He et al., 2015). Chen et al. (2013) have also found that Cd largely accumulates in the cell walls of willow roots, which probably enhances Cd tolerance. Different plants have different levels and/or or different types of tolerances to Cd stress; also different genotypes of the same species may respond differently to Cd toxicity (Di Toppi and Gabbrielli, 1999; Wu et al., 2003; Di Baccio et al., 2014). Several woody plants, such as *Populus* species, have been proposed for phytoremediation, because of their fast growth, large biomass and short rotation properties (Chen et al., 2013). Therefore, they are regarded as promising candidates for remediating Cd-contaminated soils.

Populus species are characteristically dioecious and express secondary sexual dimorphism (vegetative traits) (Juvany and Munné-Bosch, 2015). It has been generally assumed that sex-related differences in secondary sexual dimorphism are derived from reproductive cost differences (Juvany and Munné-Bosch, 2015). Females allocate more resources to reproduction than males due to the production of flowers, fruits and seeds

(Randriamanana et al., 2014). In contrast, males typically allocate more resources to vegetative growth and tolerance to abiotic stress (Chen et al., 2010; Randriamanana et al., 2014). In our recent studies, we have shown that females and males of *Populus* species have differences in growth and physiological responses to abiotic stresses, females having a higher biomass but lower tolerance when compared to males (Xu et al., 2008; Chen et al., 2010).

In this study, we employed *Populus cathayana* as a test tree species to examine the physiological and molecular mechanisms functioning under Cd stress. Based on our previous study, we know that males have a higher tolerance to Cd stress when compared to females (Chen et al., 2016). However, the sexually different response mechanisms to Cd stress in *P. cathayana* are not known well. Therefore, this study aimed to gain insight into how *P. cathayana* females and males respond to Cd stress. We hypothesize that males have superior Cd tolerance and detoxification strategies when compared to females. We intended to address the physiological and molecular mechanisms based on Cd uptake, translocation and cellular location, and to answer whether effective Cd sequestration and localization in cells and tissues are responsible for Cd tolerance and detoxification. This study not only helps us to develop better understanding of the physiological and molecular mechanisms of dioecious woody plants in response to Cd stress, but it will also enhance knowledge of the phytoremediation capacities of woody plants.

2. Materials and Methods

2.1. Plant material and experimental design

P. cathayana cuttings were collected from 60 different trees (30 males and 30 females) obtained from 15 populations in the riparian and valley flat habitats in the Qinghai Province of China (30°67′N, 104°06′E). The cuttings were cultivated as described previously by Chen et al. (2016). In brief, healthy cuttings were chosen and replanted in a greenhouse at the Hangzhou Normal University. After 4 weeks, after sprouting, the same-size cuttings were transferred into 10-L plastic pots with 10 kg of growing mixture (sand:vermiculite:perlite, 1:1:1). The plants were irrigated every three days with the nutrient solution as follows (μM, pH 6.0): 500 KCl, 900 $CaCl_2$, 300 $MgSO_4$, 600 KH_2PO_4, 42 K_2HPO_4, 2000 NH_4NO_3, 25 Fe-EDTA, 10 H_3BO_3, 0.5 $MnSO_4$, 0.5 $ZnSO_4$, 0.1 $CuSO_4$, and 0.1 $(NH_4)_6Mo_7O_{24}$.

After the plantlets were grown for further 30 d, 24 uniform cuttings were treated with either 0 (12 plants) or 50 μM $CdCl_2 \cdot 2.5\ H_2O$ (12 plants) for 120 d. The experiment included four treatments and was replicated six times, including two sexes (females and males) and two treatments (0 or 50 μM $CdCl_2 \cdot 2.5\ H_2O$). One single plant represents an independent replicate.

2.2. Gas exchange and chlorophyll fluorescence measurements

After Cd treatment for 120 d, the fourth fully developed young leaves of plants in each treatment were selected to measure gas exchange and chlorophyll fluorescence. Net CO_2 assimilation photosynthesis rate (*A*), intercellular CO_2 concentration (*Ci*), stomatal conductance (g_s) and transpiration rate *(E*) were measured with a portable photosynthesis measuring system (Ll-6400), as described previously by Chen et al. (2011). Stomatal limitation (*Ls*) was calculated as follows: L_s = 1-(C_i-C_a), where C_i is the internal leaf CO_2 concentration and C_a is the ambient CO_2 concentration (Lu et al., 2016). The chlorophyll fluorescence was determined using the PAM chlorophyll fluorometer (PAM 2100, Walz, Effeltrich, Germany), as described previously by Han et al. (2013), including *Fv/Fm*, maximum quantum efficiency of PS II photochemistry; *Y(II)*, quantum yield of photochemical energy conversion in PS II; *ETR*, electron transport rate; *Y(NO)*, quantum yield of non-regulated non-photochemical energy loss in PS II; *Y(NPQ)*, quantum yield of regulated non-photochemical energy loss in PS II; *NPQ*, non-photochemical quenching parameter describing regulated dissipation of excess energy; *qP*, photochemical quenching coefficient; *qN*, non-photochemical quenching photochemistry. The chlorophyll fluorescence measurements were conducted with three replicates for each treatment.

2.3. Determination of alondialdehyde (MDA), superoxide anion $(O_2)^-$ and hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) accumulation in roots and leaves was visualized at the subcellular level by $CeCl_3$ using the transmission electron microscope, as described by Sun et al. (2017). Briefly, fresh samples were cut into pieces and then incubated in 5 mM $CeCl_3$ (dissolved in 50 mM MOPS) for 1 h. After washing with deionized water three times, all samples were fixed in 4% glutaraldehyde (v/v) overnight. After rinsing three times with PBS (pH 7.0), samples were dehydrated with a series of ethanol solutions (30%, 50%, 70%, 80%, 90% and 95%) followed by acetone. After infiltration in Spurr resin solution, samples were sliced, stained and observed with the transmission electron microscope. The determination of MDA, O_2^- and H_2O_2 concentration in roots and leaves was conducted spectrophotometrically, as described by Chen et al. (2011). The measurements were conducted with four replicates for each treatment.

2.4. Determination of antioxidants and antioxidative enzyme activities

The activities of catalase (CAT) (EC 1.11.1.6), peroxidase (POD) (EC 1.11.1.6), glutathione reductase (GR) (EC 1.6.4.2) and superoxide dismutase (SOD) (1.15.1.1) were determined, as described by Chen et al. (2011), the total ascorbate (TA) and dehydroascorbate (DHA) were measured, as described by He et al. (2015), and GSH and oxidized glutathione (GSSG) were analyzed according to Loggini et al. (1999). Four samples from each treatment were randomly chosen to estimateCAT, POD, GR, SOD, ascorbate and DHA in leaves and roots. The measurements were conducted with four replicates for each treatment.

2.5. Determination of Cd element

Fresh roots, stems and leaves were washed with deionized water thoroughly, and dried at 75 ºC until they reached a constant weight. Dried samples were ground finely and dissolved in 3:1 (v/v) of HNO_3 and $HClO_4$. The Cd content was determined using a multi-element inductively coupled plasma mass spectrometry (Thermo X Series II, Thermo Fisher Scientific, USA). The measurements were conducted for different plants, with four replicates for each treatment.

2.6. Measurements of net Cd^{2+} fluxes in roots

Segments of about 2 cm from the tips of roots were selected to monitor Cd^{2+} fluxes using a noninvasive micro-test technique (NMT) (the NMT system BIO-IM; Younger Corp., Falmouth, MA, USA) according to the method of He et al. (2015). The root samples (control and Cd stress) were rinsed with deionized water, and then transferred into 10 mL measuring solutions (0.05 mM $CdSO_4$, 0.25 mM NaCl, 0.05 mM KCl, 0.1 mM Na_2SO_4, pH 6.0). After roots immobilized on the bottom, root ion flux measurements were performed from the apex. The measurements were conducted for different plants, with four replicates for each treatment.

2.7. Microscopic imaging of Cd localization in roots, stems, leaf blades and veins

After rinsing with distilled water, samples of roots, stems and leaves were cut into sections and freeze-dried by a vacuum freeze dryer (FreeZone 2.5, Labconco, USA) for 72 h. The location of Cd in the tissues was observed with the energy-didpersive X-ray analysis and scanning transmission electron microscopy (SEX) imaging (Sigma 500, Zeiss, Germany) equipped with an energy dispersive X-Ray spectroscopy (EDX) detector (EDAX-element, Falcon, USA). Briefly, the surface of samples was exposed to vacuum sputtering, and then the images were obtained with a scanning electron microscope at 3 kV voltage and 100 mA. The EDX spectra with S, P and Cd Kα (λ=2.3075 for S, 2.0134 for P and 23.17 for Cd) were acquired at a scanning rotation of 283.2°. After that, the line scanning was conducted with EDX at 10 kV voltage with a work distance of 8.5 mm. The measurements were conducted for different plants with four replicates for each treatment.

2.8. RNA sequencing

Total RNA was extracted by Agilent RNA 6000 Nano Kit (Agilent, USA) according to the manufacturer's instructions. RNA was assessed and purified using the NanoDropTM spectrophotometer (Thermo Fisher Scientific, USA) and Agilent 2100

Bioanalyzer (Agilent, USA). The cDNA libraries were constructed with the BGISEQ-500 platform, following a probe-anchor synthesis sequence method (BGI, China). The first cDNA was synthesized using reverse transcriptase, and the second cDNA was synthesized with DNA polymerase I and RNase H. The cDNA libraries were amplified and then quantified by Qubit® 2.0 Fluorometer (Life Technologies, USA). The ssDNA circles were used to produce DNA nanoballs to enhance the fluorescence signal, followed by sequencing using the BGISEQ-500 platform (Huada, China). Leaf and root materials were from three different plants from each treatment.

2.9. Annotation and GO enrichment analysis

The reads were aligned to the reference genome sequences of *P. trichocarpa* (http://plants.ensembl.org/Populus_trichocarpa/Info/Index). The mapping reads were constructed with StringTie software (version 1.04). The coding sequence of *P. trichocarpa* was blasted against the closest *Arabidopsis* homolog (AGI identification) with PoplarGene (http://bioinformatics.caf.ac.cn/PoplarGene/gene), and annotated using the Arabidopsis Information Resource genome (https://www.arabidopsis.org/). We used AgriGo (version 2.0) and David (version 6.8) to assign the differentially expressed genes according to the GO classification.

2.10. Statistical analysis

Statistical analyses were performed using the SPSS software (version 22.0). All data were checked for normality before analyses of variance (ANOVAs). Differences between means were analyzed by Duncan's tests following two-way ANOVAs to evaluate sex and Cd stress effects. All statistical tests were considered significant at *P*

< 0.05.

3. Results

3.1. Leaf gas exchange and fluorescence characteristics

Cd stress significantly decreased *A* in both sexes, especially in females (Table 1). No significant differences in g_s and *Trm* were detected under Cd stress when compared to the controls, with males showing higher *A*, g_s and *Trm* than females irrespective of the Cd treatment (Table 1). Cd stress significantly reduced *Ls* in females but not in males when compared to the controls. In addition, females showed lower *Fv/Fm*, *Y (II)*, *Y (NO)*, *qP* and *ETR* under Cd stress, and higher *Y (NPQ)*, *NPQ* and *qN* when compared to the controls (Table 2). In contrast, there was no significant difference in *Fv/Fm*, *Y (NO)*, *NPQ* and *qN* in males between Cd stress and control conditions. Cd stress significantly increased *Y (NPQ)* and *qN*, but reduced *Y (II)* and *ETR* in males compared to the controls.

3.2. Net Cd^{2+} fluxes in male and female roots

To examine Cd uptake, NMT was employed to monitor Cd fluxes in roots. As shown in Fig. 1, higher Cd^{2+} fluxes were detected in the apical zones of roots in both sexes under control conditions, but females had higher net Cd^{2+} fluxes than males irrespective of the Cd treatment. As shown in Fig. 1, females had slightly higher Cd levels in roots and much higher levels in leaves when compared to males, while male stems accumulated more Cd than those of females.

3.3. Cd sequestration in male and female roots, stems and leaves

Cd distribution was monitored across leaf blades. Males showed a higher Cd signal intensity, especially in the epidermis when compared to females (Fig. 2). Furthermore, the highest Cd signal intensity of males was mainly in the upper rather than lower epidermis, while the opposite was true for females (Fig. 2). Males possessed higher Cd signals in epidermal, vascular and spongy tissues, while more Cd sequestrated into the palisade tissues in females. In leaf veins, females had far greater Cd signals than males (Fig. 2). In both sexes, more Cd was allocated to the epidermis, which belonged to the abaxial rather than to the adaxial surface of leaves. However, males showed more Cd in the cortical tissues, while in females the Cd signal located mainly in the vascular tissues. In roots, Cd preferred to gather in the vascular tissues in females, while males had strongest Cd signals in epidermal and cortical tissues (Fig. 3). In stems, males had stronger Cd signals than females (Fig. 3). Cd location patterns were closely similar in the two sexes, although Cd signals were higher in males than in females. In both sexes, Cd favored the pith and vascular tissues in stem cross-sections.

3.4. Correlation of Cd with P and S

As shown in Fig. 4, the positive correlation between Cd and S was much higher in males than in females ($R^2=0.21$, females; $R^2=0.62$, males). Cd and P did not correlate significantly in females ($R^2=0.013$), while in males they did ($R^2=0.47$). Furthermore, TEM observations and the EDX analysis revealed that P signals across female leaf blades and veins were significantly higher than those of S irrespective of Cd (Figs. S1-S4). In contrast, in males, S signals were higher relative to P in the control plants, whereas Cd stress elevated the proportion of P in leaf veins but not in blades. The proportions of P and S across root cross-sections were almost similar irrespective of Cd stress in both sexes.

3.5. ROS and antioxidants

As shown in Fig. 5, Cd exposure induced H_2O_2 production in the leaves and roots of both females and males, and there was no significant difference between the sexes irrespective of Cd stress. The histochemical visualization of H_2O_2 in roots and leaves revealed considerable H_2O_2 accumulation in the cell walls of both sexes under Cd stress (Fig. 5b). Moreover, MDA and O_2^- increased significantly in female leaves and roots under Cd stress, while Cd stress did not affect MDA in male leaves and roots. O_2^- increased in male roots treated with Cd but not in leaves (Fig. 5c, d).

When compared to control conditions, Cd stress significantly reduced CAT, SOD and GR activities, by 36%, 28% and 34% in leaves, and by 65%, 63% and 52% in roots, respectively. In males, there was no significant change in leaf CAT and GR, and in root SOD activities under Cd stress when compared to the controls. Yet, Cd stress significantly increased root CAT, GR and POD activities, and leaf SOD and POD activities in males when compared to the controls (Fig. S5). We further examined the levels of antioxidants in leaves. The total glutathione (GSSG+GSH) and GSH levels were far higher in males than in females under Cd stress (Fig. 5e). The ratio of GSH to GSSG was also higher in males but not in females when compared to the controls. The total ascorbate and ascorbate levels increased in both sexes under Cd stress, and Cd stress increased the ratio of ASC to DHA in males but not in females (Fig. 5f).

3.6. Transcriptome dynamics in response to Cd stress in females and males

As shown in Fig. S6, 1802 and 750 common DEGs between FC (Female-Cd) and FCd

(Female+Cd), 3849 and 3863 ones between MC (Male-Cd) and MCd (Male+Cd), and 3068 and 340 ones between “FC versus FCd” and “MC versus MCd” were identified in roots and leaves, respectively. Hierarchical clustering of differently expressed genes in leaves and roots were performed according to their relative transcript abundance (Fig. 6, Tables S1-S6). According to differences in expression patterns between sexes and Cd treatments, the modes of DEGs and corresponding annotations for homologs to Arabidopsis were further analyzed with agriGO and David.

As shown in Fig. 6, the gene expressions of module G2 between “FC vs FCd” in leaves were sex-dependent and affected by Cd stress. The GO enrichment found that module G2 between “FC vs FCd” in leaves was enriched in the cell wall organization, ATPase activity and transcript regulation. By contrast, the upregulation of genes between MC and MCd in leaves was focused on the secondary metabolism, responses to hormone stimulus and stress, secondary metabolism, and vacuole and cell wall processes. In roots, Cd stress induced upregulation of genes in females but not in males. Genes in the module G2 between “FC vs FCd” were enriched for oxidation reduction, transition metal ion binding, plasma membrane, responses to abiotic stress, carbohydrate transport, ABC transport, ATPase activity and defense responses. The only downregulated genes by Cd in the roots of females were enriched in the module G3. To further categorize the functions of DEGs between sexes and Cd treatment, DEGs were blasted against the Arabidopsis homologs (AGI identification). We found that the gene expressions in the module G3 between “FC vs FCd” were closely correlated with root development, cell wall structure, auxin signaling pathway and water channel activity

(Fig. 6). By contrast, the upregulated genes induced by Cd stress in males were related to secondary metabolic processes, lignin metabolic processes, responses to hormone and abiotic stimulus, oxidation reduction, disulfide bond and flavonoid biosynthesis processes, and vacuole membrane.

We queried the sexually differential expressed genes by exploring gene functions and enrichment under control and Cd stress conditions (Fig. S7; Tables S7-S12). Most up-regulated genes in leaves between "FC vs MC" under control conditions were related to flavonol biosynthesis and metabolism, ATP binding, protein phosphorylation, glutathione metabolism and detoxification. Most down-regulated genes were connected to photosynthesis, cell division, fatty acid metabolism, amino acid transport and auxin signaling pathway (Fig. S7). Stress response, ATPase activity and ABC transport functions were enriched in both sexes under Cd stress. When exposed to Cd stress, the higher gene expression levels in female leaves relative to males concerned oxidation-reduction processes, microtubules, extracellular signal peptides, auxin biosynthesis and GTPase activity, while males had higher gene expression levels related to oxidation-reduction processes, iron binding, secondary metabolite biosynthesis and water channel activity (Fig. S7).

In roots, the upregulated genes between "FC vs MC" under control conditions related to oxidation-reduction, gibberellin biosynthesis, potassium transport, responses to ethylene and salicylic acid, and cell wall, while the downregulated genes concerned ATP binding, kinase activity, flavonoid biosynthesis and ABC transport (Fig. S7). Cd stress significantly elevated the processes of oxidoreductase, secondary metabolism and cell wall biogenesis in males relative to females. In contrast, genes functionally related to transmembrane, ion transport and water channel activity displayed higher expression in female roots than in male roots. The processes of oxidoreductase and metabolism in female roots were also found enhanced in females, but they had lower enrichment scores when compared to males under Cd stress.

3.7. Transcriptome dynamics related to Cd uptake, translocation and detoxification

Females and males displayed sexual difference in Cd tolerance and allocation among tissues. Thus, transcriptional changes in genes involved in Cd uptake, transport and detoxification were expected. As shown in Fig. 7, we found that genes related to Cd detoxification *PCS* and *ABCC1* were upregulated in male leaves under Cd stress, while the genes encoding plasma membrane H^+-ATPase *VHA1.1* and *HA2.1* were downregulated in male leaves. There was no significant difference in these gene expressions under Cd stress and control conditions in female leaves. In roots, genes related to Cd transport, *ZIP2*, *ZIP11*, *YSL7* and *NRT1.1,* were significantly upregulated by Cd stress in females, but not in males. Moreover, genes related to Cd detoxification, *MTP1* and *ABCC1,* were upregulated in roots of both sexes, especially in males.

4. Discussion

4.1. Cd uptake, allocation and sequestration in males led to a superior tolerance when compared to females

Heavy metals disturb plant growth and metabolism, but the effects differ among plant species and between sexes (DalCorso et al., 2008; Gallego et al., 2012; Meyer et al., 2015). As the first checkpoint, roots play a vital role in Cd transport and accumulation.

In root tips, especially the apical zones are regarded as the main position for Cd^{2+} uptake because of dysplasia or lack of epidermal cell wall (Chen et al., 2013). Consistently, we found that high net Cd^{2+} influx was detected in root apex in both sexes irrespective of the Cd treatment, although the highest levels were found in female roots under control conditions (Fig. 1). Cd absorbed by roots is sequestrated into cellular compartments and transported upwards to other plant parts through xylem loading via the transpiration stream (Wu et al., 2015; Liu et al., 2016). In this study, males had higher Cd accumulation in stems but lower Cd in leaves when compared to females (Fig. 1). This could be explained by strong Cd sequestration into cell walls and/or vacuoles in male roots and stems. Additionally, Cd preferred to gather in the vascular tissues of females, thus facilitating the accumulation of Cd in leaves. Our results suggested that females have a higher capacity for Cd uptake and root-to-shoot translocation when compared to males.

The effective sequestration of Cd into the stem has been regarded as a preferential strategy of heavy metal detoxification in plants (Tian et al., 2017). Bark was recently found to serve as a large storage site for Cd sequestration, as shown for phloem in *P.* × *canescens* (He et al., 2013). Cd is preferentially stored in metabolically less active cellular compartments, including epidermis, and cortical and vascular tissues of stems (Tian et al., 2017). Our results suggested that male stems accumulated more Cd than those of females (Fig. 1). Cd was found to preferentially sequestrate into epidermis and cortical tissues, and also to male bark, which presumably protects leaves against Cd damage (He et al., 2013). After transporting in the phloem, Cd reaches leaves and begins to enrich. Similarly to the spatial distribution of heavy metals in leaves, Cd predominantly accumulates in the epidermis and vascular bundles, and less in the mesophyll (Tian et al., 2017). Consistently, Cd-tolerant males preferred to allocate more Cd into leaf veins and less into the mesophyll compared to females (Figs 2-3). Males also preferred to allocate more Cd to epidermis and cortex compared to females, especially to the upper rather than to the lower epidermis (Fig. 2), which helped to protect the stomatal apparatus from Cd damage. Mesophyll appears to be a vital sink for Cd, since it represents a higher biomass volume than the epidermal tissues (Galiová et al., 2019).

Our study showed that males preferentially allocated Cd to the spongy tissues, while in females the leaf palisade tissues accumulated more Cd (Fig. 2). The palisade tissues play an important role in photosynthesis due to their structure and function (Gotoh et al., 2018). Consistently, in response to Cd stress, males had a higher photosynthesis capacity and only little damage to the electron transport and PSII reaction centers when compared to females (Tables 2-3). Overall, males had more effective strategies than females, that is, preferential Cd sequestration into cellular compartments of less metabolically active tissues, to cope with Cd toxicity.

4.2. The correlations between P, S and Cd partly explained the sexual difference in Cd tolerance

In this study, S distribution was positively related to Cd in both sexes, but more strongly in males. A positive correlation was detected between P and Cd in males but not in females (Fig. 4). These results suggested that P and S probably played a vital role in the

Cd tolerance of males. For example, the use of P fertilizers reduces Cd availability to plants via reducing Cd mobility in soil, as well as neutralizes Cd toxicity by a diluting effect (Dheri et al., 2007; Matusik et al., 2008; Bolan et al., 2003). P is involved in the phytochelatin biosynthesis, which transports Cd into vacuoles by Cd/phytochelatin complexes (Salt and Rauser, 1995). Cd was also chelated with phosphates and then sequestrated into cell walls (Parrotta et al., 2015). Therefore, the positive correlation between Cd and P probably partly explained the Cd tolerance of males. The alleviating effect of S on Cd toxicity has been reported previously as well (Ding et al., 2017; Matraszek et al., 2017; Baig et al., 2019). S enhances GSH synthesis, which is regarded as an important defensive response against metal toxicity (Liang et al., 2016). In this study, males had a greater ratio of GSH to GSSH than females in leaves, thus further demonstrating the role of S in Cd detoxification.

4.3. Sex-specific molecular mechanism under Cd stress

Females and males show significant sexual differences in phenology, growth and physiology in responses to abiotic stress, and females often exhibit a lower tolerance than males (Chen et al., 2011; Chen et al., 2016). In this study, we investigatedd the physiological and molecular mechanisms in males and females with or without Cd stress. The transcriptional data revealed a sex-specific mechanism. Females appeared to allocate more resources to growth, since most upregulated genes in leaves were involved in photosynthesis, cell division and ubiquitin-protein ligase binding (Fig. S7). By contrast, males allocated more energy into defense, since most upregulated genes in leaves were related to flavonoid metabolism, ATPase activity and disulfide bond (Fig.

S7). In roots, the upregulated genes were involved in transmembrane transport and energy metabolism of female roots, thus indicating that females needed more resources to maintain growth and development.

Sex-related differences in gene expression patterns are probably related to differences in reproductive costs (Juvany and Munné-Bosch, 2015). Females allocate more resources to reproduction than males (Juvany and Munné-Bosch, 2015). The maintenance of reproductive vigor usually limits vegetative growth and defense investment (Graff et al., 2013; Juvany and Munné-Bosch, 2015). In the present study, we observed that sexually differential gene expression could be weakened by Cd stress (Fig. S7). Notably, the common expressed genes in leaves and roots between “FC vs MC” and “FCd vs MCd” had similar expression patterns. The common upregulated genes in the leaves between “FC vs MC” and “FCd vs MCd” were oxidoreductase, secondary metabolite and glutathione metabolism –related genes, and the downregulated ones were transmembrane, ABC transmembrane transport and protein phosphorylation –related genes. These results indicated that females and males displayed specific patterns in growth and physiology, also at the molecular level.

4.4. Molecular mechanism underlying sexually different regulation in Cd uptake, sequestration (translocation) and tolerance

Transcriptional profiles have been utilized to study sexual differences at the molecular level in response to Cd stress in *P. cathayana*. Cd is a non-essential element for plants, generally transported into root cells accompanied by other cations, such as Zn, Ca and Fe. Some proteins belonging to the ZIP family, NRAMP, CDF and CPx-ATPase are involved in Cd transport from soil (Yu et al., 2018). Most up-regulated genes in female roots induced by Cd stress were involved in transport processes, such as *YELLOW STRIPE LIKE 7*, *Zinc transporter 2*, *Zinc transporter 11* and *cation exchanger 3* (Figs 6-7). Nitrate transporters have recently been reported to be involved in Cd uptake and tolerance (Li et al., 2010; Mao et al., 2014). In female roots exposed to Cd, nitrate transporter *NRT1;1* was significantly upregulated. Nitrogen, sulfur and phosphorus have been found to be involved in Cd detoxification in plants (Sarwar et al., 2010). The up-regulation of ammonium transporter *AMT2*, *phosphate transporter 1;7* and *sulfate transporter 1;3* taking place in female roots under Cd exposure indicated that such genes probably have a role in the response of females to Cd stress (Figs 6-7; Tables S1-S6).

Noticeably, the sensitivity of females to Cd stress probably resulted in a greatly reduced gene expression related to metabolism, antioxidation and GSH biosynthesis (Fig. 6). It was noteworthy that the downregulation of genes in female roots was enhanced related to root growth, auxin signaling and cell morphology, indicating that females were more sensitive to Cd stress than males. The upregulation of genes encoding ion transporters in females, especially for Cd transport, probably led to greater Cd flux into roots, which in turn further increased Cd toxicity. By contrast, we only screened the upregulated genes, which were affected by Cd and only expressed in male roots; most of these gene expressions were involved in secondary metabolism, responses to abiotic stress and hormone stimulus, as well as lignin biosynthesis and metabolism. Stimulation of lignin synthesis probably played a role in Cd detoxification, as documented in other studies

(Ma et al., 2014).

Effective Cd sequestration in cell walls and vacuoles facilitates Cd detoxification in plants (Sharma et al., 2016; Peng et al., 2017). Consistently, we found that genes related to wall biosynthesis and metabolism, such as *wall assocated kinase–like WAKL1* and *WAKL2*, *xylem cysteine peptidase XCP1*, *xylem serine peptidase XSP1*, *xyloglucan endotransglycosylase XTH2* and *XTR6*, and *cellulose synthase-like B4* were significantly induced by Cd stress in male roots, indicating that the cell wall is probably involved in Cd sequestration. Cd sequestration in cell walls also reduces the root-to-shoot translocation of Cd (Li et al., 2015). Metal tolerance genes *MTP3* were also upregulated by Cd stress in the vacuoles of male roots. Importantly, genes encoding responses to hormones, such as ABA, ethylene, auxin and gibberellin, were induced by Cd only in male roots.

It has been widely reported that ABA, auxin and gibberellin play important roles in alleviating heavy metal stress, including Cd stress (Alves et al., 2017; Bücker-Neto et al., 2017). In addition, some secondary products, phenolic glycosides and flavonoids elevate plants' tolerance to Cd stress (Shakirova et al., 2016; Berni et al., 2019). The better Cd tolerance of males appeared to be also correlated with secondary metabolism, since most genes related to responses to jasmonic acid, salicylic acid and flavonoid metabolism were significantly upregulated in males (Fig. 6). Cd sequestration into either vacuoles or cell walls has been found to facilitate reduced Cd translocation to shoots (Sharma et al., 2016; Luo et al., 2019). In addition, we discovered that some genes related to antioxidation were induced by Cd stress in male roots.

Females and males adopted different strategies to cope with Cd stress in leaves (Fig. 8). In males, genes related to GA metabolism, cell wall biosynthesis and antioxidative responses were significantly up-regulated by Cd stress, while genes related to catalytic activity were significantly upregulated by Cd stress in females. In addition, we found that Cd stress probably affected ion homeostasis, glutamate metabolism and jasmonate synthesis, since some genes involved in these processes were significantly up-regulated in the male leaves. These results suggested that the stronger Cd tolerance in males probably resulted from the reduced Cd accumulation in leaves and effective cellular Cd detoxification.

5. Conclusions

Our results suggested that sexual differences in responses to Cd stress in females and males are attributable to differential Cd uptake, tolerance and detoxification. Females had highly efficient root-to-shoot translocation, as showed by the presence of more extensive Cd signals in the vascular tissues of roots and stems. Moreover, the considerable Cd distribution in leaf mesophyll tissues of females may be a critical factor for their high Cd sensitivity. In contrast, males had reduced root-to-shoot translocation and effective Cd accumulation in bark. The subcellular sequestration of Cd into epidermal and cortical tissues of roots, leaves and stems probably represented a critical process in the Cd tolerance of males. Combined with the consistent distribution of P and S with Cd, this study suggested that most Cd in epidermal and cortical tissues is probably chelated by P and S in males. In addition, our transcription data also suggested that the better Cd tolerance of males is correlated with the induction of secondary metabolism, hormonal responses and subcellular detoxification of Cd. This study provided new insights into sex-specific translocation, sequestration and detoxification related to Cd tolerance in dioecious woody plants. There are further applications in the use of woody plants for phytoremediation.

Acknowledgements This work was supported by the Zhejiang Provincial Natural Science Foundation of China (LQ18C030004) and the Talent Program of the Hangzhou Normal University (2016QDL020).

Appendix A. Supplementary data

Supplementary material related to this article can be found in the supplementary information.

References

Alves, L.R., Monteiro, C.C., Carvalho, R.F., Ribeiro, P.C., Tezotto, T., Azevedo, R.A., Gratão, P.L., 2017. Cadmium stress related to root-to-shoot communication depends on ethylene and auxin in tomato plants. Environ. Exp. Bot. 134, 102-115. https://doi.org/10.1016/j.envexpbot.2016.11.008.

Baig, M.A., Ahmad, J., Ali, A.A., Amna, Qureshi, M.I., 2019. "Role of Sulfur Metabolism in Cadmium Tolerance." Cadmium Tolerance in Plants. Academic Press, pp. 335-365.

Berni, R., Luyckx, M., Xu, X., Legay, S., Sergeant, K., Hausman, J.F., Lutts, S., Cai, G., Guerriero, G., 2019. Reactive oxygen species and heavy metal stress in plants: impact on the cell wall and secondary metabolism. Environ. Exp. Bot. 161, 98-106. https://doi.org/10.1016/j.envexpbot.2018.10.017.

Bi, J.W., Liu, X.C., Liu, S.R., Wang, Y.T., Liu, M., 2020. Microstructural and physiological responses to cadmium stress under different nitrogen forms in two contrasting *Populus* clones. Environ. Exp. Bot. 169, 103897. https://doi.org/10.1016/j.envexpbot.2019.103897.

Bolan, N.S., Adriano, D.C., Duraisamy, P., Mani, A., Arulmozhiselvan, K., 2003. Immobilization and phytoavailability of cadmium in variable charge soils. II. Effect of phosphate addition. Plant Soil 250, 83-94. https://doi.org/10.1023/A:1022826014841.

Bücker-Neto, L., Paiva, A.L.S., Machado, R.D., Arenhart, R.A., Margis-Pinheiro, M., 2017. Interactions between plant hormones and heavy metals responses. Genet. Mol. Biol. 40, 373-386. http://dx.doi.org/10.1590/1678-4685-gmb-2016-008.

Chen, G., Liu, Y., Wang, R., Zhang, J., Owens, G., 2013. Cadmium adsorption by willow root: the role of cell walls and their subfractions. Environ. Sci. Pollut. Res. 20, 5665-5672. https://doi.org/10.1007/s11356-013-1506-3.

Chen, J., Duan, B., Xu, G., Korpelainen, H., Niinemets, Ü., Li, C., 2016. Sexual competition affects biomass partitioning, carbon-nutrient balance, Cd allocation and ultrastructure of *Populus cathayana* females and males exposed to Cd stress. Tree Physiol. 36, 1353-1368. https://doi.org/10.1093/treephys/tpw054.

Chen, L.H, Han, Y., Jiang, H., Korpelainen, H., Li, C.Y., 2011. Nitrogen nutrient status induces sexual differences in responses to cadmium in *Populus yunnanensis*. J. Exp. Bot. 62, 5037-5050. https://doi.org/10.1093/jxb/err203.

Chen, L.H., Zhang, S., Zhao, H.X., Korpelainen, H., Li, C.Y., 2010. Sex-related adaptive responses to interaction of drought and salinity in *Populus yunnanensis.* Plant Cell Environ. 33(10), 1767-1778. https://doi.org/10.1111/j.1365-3040.2010.02182.x.

DalCorso, G., Farinati, S., Maistri, S., Furini, A., 2008. How plants cope with cadmium: staking all on metabolism and gene expression. J. Integr. Plant Biol. 50(10), 1268-1280. https://doi.org/10.1111/j.1744-7909.2008.00737.x.

Dheri, G.S., Singh Brar, M., Malhi, S.S., 2007. Influence of phosphorus application on growth and cadmium uptake of spinach in two cadmium-contaminated soils. J. Plant Nut. Soil Sci. 170, 495-499. https://doi.org/10.1002/jpln.200625051.

Di Toppi, L.S., & Gabbrielli, R. 1999. Response to cadmium in higher plants. Environ. Exp. Bot. 41(2), 105-130. https://doi.org/10.1016/S0098-8472(98)00058-6.

Di Baccio, D., Castagna, A., Tognetti, R., Ranieri, A., Sebastiani, L. 2014. Early responses to cadmium of two poplar clones that differ in stress tolerance. J. Plant physiol. 171(18), 1693-1705. https://doi.org/10.1016/j.jplph.2014.08.007.

Ding, S., Ma, C., Shi, W., Liu, W., Lu, Y., Liu, Q., Luo, Z.B., 2017. Exogenous glutathione enhances cadmium accumulation and alleviates its toxicity in *Populus* × *canescens*. Tree Physiol. 37, 1697-1712. https://doi.org/10.1093/treephys/tpx132.

Galiová, M.V., Száková, J., Prokeš, L., Čadková, Z., Coufalík, P., Kanický, V., Otruba, V., Tlustoš, P., 2019. Variability of trace element distribution in *Noccaea* spp., *Arabidopsis* spp., and *Thlaspi arvense* leaves: the role of plant species and element accumulation ability. Environ. Monit. Assess. 191(3), 181. https://doi.org/10.1007/s10661-019-7331-5.

Gallego, S.M., Pena, L.B., Barcia, R.A., Azpilicueta, C.E., Iannone, M.F., Rosales, E.P., Zawoznik M.S., Groppa M.D., Benavides, M.P., 2012. Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. Environ. Exp. Bot. 83, 33-46. https://doi.org/10.1016/j.envexpbot.2012.04.006.

Graff, P., Rositano, F., Aguiar, M.R., 2013. Changes in sex ratios of a dioecious grass with grazing intensity: the interplay between gender traits, neighbor interactions and spatial patterns. J. Ecol. 101, 1146-1157. https://doi.org/10.1111/1365-2745.12114.

Gotoh, E., Suetsugu, N., Higa, T., Matsushita, T., Tsukaya, H., Wada, M., 2018. Palisade cell shape affects the light-induced chloroplast movements and leaf photosynthesis. Sci. Rep. 8, 1472. https://doi.org/10.1038/s41598-018-19896-9.

Gupta, D.K., Pena, L.B., Romero-Puertas, M.C., Hernández, A., Inouhe, M., Sandalio, L.M., 2017. NADPH oxidases differentially regulate ROS metabolism and nutrient uptake under cadmium toxicity. Plant Cell Environ. 40, 509-526. https://doi.org/10.1111/pce.12711.

Han, Y., Wang, L., Zhang, X., Korpelainen, H., Li, C., 2013. Sexual differences in photosynthetic activity, ultrastructure and phytoremediation potential of *Populus* *cathayana* exposed to lead and drought. Tree Physiol. 33, 1043-1060. https://doi.org/10.1093/treephys/tpt086.

Hasan, M.K., Ahammed, G.J., Yin, L.L., Shi, K., Xia, X.J., Zhou, Y.H., Yu, J.Q., Zhou, J., 2015. Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatins biosynthesis, vacuolar sequestration, and antioxidant potential in *Solanum lycopersicum* L. Front Plant Sci. 6, 601. https://doi.org/10.3389/fpls.2015.00601.

He, J.L., Li, H., Luo, J., Ma, C.F., Li, S.J., Qu, L., Gai, Y., Jiang, X.N., Janz, D., Polle, A., Tyree, M., 2013. A transcriptomic network underlies microstructural and physiological responses to cadmium in *Populus* × *canescens*. Plant Physiol. 162, 424-439. https://doi.org/10.1104/pp.113.215681.

He, J.L., Li, H., Ma, C.F., Zhang, Y.L., Polle, A., Rennenberg, H., Cheng, X.Q., Luo, Z.B., 2015. Overexpression of bacterial γ-glutamylcysteine synthetase mediates changes in cadmium influx, allocation and detoxification in poplar. New Phytol. 205, 240-254. https://doi.org/10.1111/nph.13013.

Huang, R.Z., Jiang, Y.B., Jia, C.H., Jiang, S.M., Yan, X.P., 2018. Subcellular distribution and chemical forms of cadmium in *Morus alba* L. Int. J. Phytoremediat. 20(5), 448-453. https://doi.org/10.1080/15226514.2017.1365344.

Juvany, M., Munné-Bosch. S., 2015. Sex-related differences in stress tolerance in dioecious plants: a critical appraisal in a physiological context. J. Exp. Bot. 66, 6083-6092. https://doi.org/10.1093/jxb/erv343.

Li, J.Y., Fu, Y.L., Pike, S.M., Bao, J., Tian, W., Zhang, Y., Chen, C.Z., Zhang, Y., Li, H.M., Huang, J. et al.et al., 2010. The *Arabidopsis* nitrate transporter *NRT1. 8* functions in nitrate removal from the xylem sap and mediates cadmium tolerance. Plant Cell 2, 1633-1646. https://doi.org/10.1105/tpc.110.075242.

Li, T., Tao, Q., Shohag, M.J.I., Yang, X., Sparks, D.L., Liang, Y., 2015. Root cell wall polysaccharides are involved in cadmium hyperaccumulation in *Sedum alfredii*. Plant Soil 389(1-2), 387-399. https://doi.org/10.1007/s11104-014-2367-3.

Liang, T., Ding, H., Wang, G., Kang, J., Pang, H., Lv, J., 2016. Sulfur decreases cadmium translocation and enhances cadmium tolerance by promoting sulfur assimilation and glutathione metabolism in *Brassica chinensis* L. Ecotoxicol. Environ. Safety 124, 129-137. https://doi.org/10.1016/j.ecoenv.2015.10.011.

Liu, H., Zhao, H., Wu, L., Liu, A., Zhao, F.J., Xu, W., 2017. Heavy metal ATPase 3 (HMA3) confers cadmium hypertolerance on the cadmium/zinc hyperaccumulator *Sedum plumbizincicola*. New Phytol. 215, 687-698. https://doi.org/10.1111/nph.14622.

Liu, H.W., Wang, H.Y., Ma, Y.B., Wang, H.H., Shi, Y., 2016. Role of transpiration and metabolism in translocation and accumulation of cadmium in tobacco plants (*Nicotiana tabacum* L.). Chemosphere 144, 1960-1965. https://doi.org/10.1016/j.chemosphere.2015.10.093.

Loggini, B., Scartazza, A., Brugnoli, E., Navari-Izzo, F., 1999. Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol. 119(3), 1091-1100. https://doi.org/10.1104/pp.119.3.1091.

Lu, Z., Lu, J., Pan, Y., Lu, P., Li, X., Cong, R., Ren, T., 2016. Anatomical variation of mesophyll conductance under potassium deficiency has a vital role in determining leaf photosynthesis. Plant Cell Environ. 39, 2428-2439. https://doi.org/10.1111/pce.12795.

Luo, J.S., Yang, Y., Gu, T., Wu, Z., Zhang, Z., 2019. The *Arabidopsis* defensin gene *AtPDF2*. 5 mediates cadmium tolerance and accumulation. Plant Cell Environ. 1–15. https://doi.org/10.1111/pce.13592.

Ma, Y., He, J., Ma, C., Luo, J., Li, H., Liu, T., Polle, A., Peng, C.H., Luo, Z.B., 2014. Ectomycorrhizas with *Paxillus involutus* enhance cadmium uptake and tolerance in *Populus*× *canescens*. Plant Cell Environ. 37(3), 627-642. https://doi.org/10.1111/pce.12183.

Mao, Q.Q., Guan, M.Y., Lu, K.X., Du, S.T., Fan, S.K., Ye, Y.Q., Lin, X.Y., Jin, C.W., 2014. Inhibition of nitrate transporter 1.1-controlled nitrate uptake reduces cadmium uptake in *Arabidopsis*. Plant Physiol. 166, 934-944. https://doi.org/10.1104/pp.114.243766.

Matraszek, R., Chwil, S., Hawrylak-Nowak, B., Kozłowska-Strawska, J., 2017. Effect of sulphur and cadmium on macronutrient balance in spring wheat. Proc. Natl. Acad. Sci. India Section B: Biol. Sci. 87, 927-936. https://doi.org/10.1007/s40011-015-0658-y.

Matusik, J., Bajda, T., Manecki, M., 2008. Immobilization of aqueous cadmium by addition of phosphates. J Hazard. Mater. 152, 1332-1339. https://doi.org/10.1016/j.jhazmat.2007.08.010.

Meyer, C.L., Juraniec, M., Huguet, S., Chaves-Rodriguez, E., Salis, P., Isaure, M.P., Goormaghtigh, N.V., Verbruggen, N., 2015. Intraspecific variability of cadmium tolerance and accumulation, and cadmium-induced cell wall modifications in the metal hyperaccumulator *Arabidopsis halleri*. J. Exp. Bot. 66, 3215-3227. https://doi.org/10.1093/jxb/erv144.

Parrotta, L., Guerriero, G., Sergeant, K., Cai, G., Hausman, J.F., 2015. Target or barrier? The cell wall of early-and later-diverging plants vs cadmium toxicity: differences in the response mechanisms. Front Plant Sci. 6, 133. https://doi.org/10.3389/fpls.2015.00133.

Peng, J.S., Wang, Y.J., Ding, G., Ma, H.L., Zhang, Y.J., Gong, J.M., 2017. A pivotal role of cell wall in cadmium accumulation in the Crassulaceae hyperaccumulator *Sedum plumbizincicola*. Mol. Plant 10, 771-774. https://doi.org/10.1016/j.molp.2016.12.007.

Randriamanana, T.R., Nybakken, L., Lavola, A., Aphalo, P.J., Nissinen, K., Julkunen-Tiitto, R. 2014. Sex-related differences in growth and carbon allocation to defence in *Populus tremula* as explained by current plant defence theories. Tree Physiol. 34(5), 471-487. https://doi.org/10.1093/treephys/tpu034.

Salt, D.E., & Rauser, W.E., 1995. MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiol. 107, 1293-1301. https://doi.org/10.1104/pp.107.4.1293.

Shakirova, F.M., Allagulova, C.R., Maslennikova, D.R., Klyuchnikova, E.O., Avalbaev, A.M., Bezrukova, M.V. 2016. Salicylic acid-induced protection against cadmium toxicity in wheat plants. Environ. Exp. Bot. 122, 19-28. https://doi.org/10.1016/j.envexpbot.2015.08.002.

Sarwar, N., Malhi, S.S., Zia, M.H., Naeem, A., Bibi, S., Farid, G., 2010. Role of mineral nutrition in minimizing cadmium accumulation by plants. J. Sci. Food Agric. 90, 925-937. https://doi.org/10.1002/jsfa.3916.

Sharma, S.S., Dietz, K.J., Mimura, T., 2016. Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. Plant Cell Environ. 39, 1112-1126. https://doi.org/10.1111/pce.12706.

Sun, C., Liu, L., Zhou, W., Lu, L., Jin, C., Lin, X., 2017. Aluminum induces distinct changes in the metabolism of reactive oxygen and nitrogen species in the roots of two wheat genotypes with different aluminum resistance. J. Agric. Food Chem. 65, 9419-9427. https://doi.org/10.1021/acs.jafc.7b03386.

Tian, S.K., Xie, R.H., Wang, H.X., Hu, Y., Hou, D.D., Liao, X.C., Brown, P.H., Yang, X.E., Liu, X.Y., Labavitch, J.M., et al.,. 2017. Uptake, sequestration and tolerance of cadmium at cellular levels in the hyperaccumulator plant species *Sedum alfredii*. J. Exp. Bot. 68, 2387-2398. https://doi.org/10.1093/jxb/erx112.

Vollenweider, P., Cosio, C., Günthardt-Goerg, M.S., 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(1-3), 25-40. https://doi.org/10.1016/j.envexpbot.2005.06.012.

Wong, C.K.E., Jarvis, R.S., Sherson, S.M., Cobbett, C.S., 2009. Functional analysis of the heavy metal binding domains of the Zn/Cd-transporting ATPase, HMA2, in

Arabidopsis thaliana. New Phytol. 181(1), 79-88. https://doi.org/10.1111/j.1469-8137.2008.02637.x.

Wu, F., Zhang, G., Dominy, P. 2003. Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. Environ. Exp. Bot. 50(1), 67-78.

Wu, Z., Zhao, X., Sun, X., Tan, Q., Tang, Y., Nie, Z., Hu, C., 2015. Xylem transport and gene expression play decisive roles in cadmium accumulation in shoots of two oilseed rape cultivars (*Brassica napus*). Chemosphere 119, 1217-1223. https://doi.org/10.1016/j.chemosphere.2014.09.099.

Xu, X., Yang, F.A.N., Xiao, X., Zhang, S., Korpelainen, H., Li, C.Y., 2008. Sex-specific responses of *Populus cathayana* to drought and elevated temperatures. Plant Cell Environ. 31(6), 850-860. https://doi.org/10.1111/j.1365-3040.2008.01799.x.

Yu, R., Ma, Y., Li, Y., Li, X., Liu, C., Du, X., Shi, G., 2018. Comparative transcriptome analysis revealed key factors for differential cadmium transport and retention in roots of two contrasting peanut cultivars. BMC Genomics 19, 938. https://doi.org/10.1186/s12864-018-5304-7.

Zhang, J., Martinoia, E., Lee, Y., 2018. Vacuolar transporters for cadmium and arsenic in plants and their applications in phytoremediation and crop development. Plant Cell Physiol. 59, 1317-1325. https://doi.org/10.1093/pcp/pcy006.

Zhao, F.J., Huang, X.Y., 2018. Cadmium phytoremediation: call rice CAL1. Mol. Plant 11, 640-642. https://doi.org/10.1016/j.molp.2018.03.016.

Figure legends

Fig. 1. Net Cd^{2+} fluxes in apical and mature zones of roots (a-c), and Cd concentrations in roots, stems and leaves (d) of *P. cathayana* females and males exposed to Cd stress. Different letters on the bars indicate significant differences between the treatments. Values are expressed as means ± SD (n = 4).

Fig. 2. Cd distribution in the leaf blade and vein cross-sections of *P. cathayana* females and males exposed to Cd stress, as determined by energy-dispersive x-ray analysis and scanning electron microscope imaging. The X-axis represents the distance between two points of leaf blade (a) and leaf vein (b) cross-sections (red lines). The Y-axis represents the relative intensity of Cd distribution on the line connecting two points of cross-sections (red lines). Leaf mesophyll upper epidermis (ue), leaf mesophyll palisade

tissue (pa), leaf spongy palisade tissue (sp), leaf lower epidermis (le), leaf vein adaxial collenchyma (adc), leaf vein abaxial collenchyma (abc), leaf vein epidermis (ep), leaf vein cortices (co), leaf vein phloem (ph), leaf vein xylem (x), leaf vein pith (p).

Fig. 3. Cd distribution in the stem and root cross-sections of *P. cathayana* females and males exposed to Cd stress, as determined by energy-dispersive x-ray analysis and scanning electron microscope imaging. The X-axis represents the distance between two points of stem (a) and roots (b) cross-sections (red lines). The Y-axis represents the relative intensity of Cd distribution on the line connecting two points of cross-sections. Epidermis (ep), cortices (co), phloem (ph), xylem (x), stem pith (p).

Fig. 4. Correlation analysis of cadmium (Cd), sulfur (S) and phosphorus (P) in *P. cathayana* females and males exposed to cadmium stress, as determined by energy-dispersive x-ray analysis and scanning electron microscope imaging.

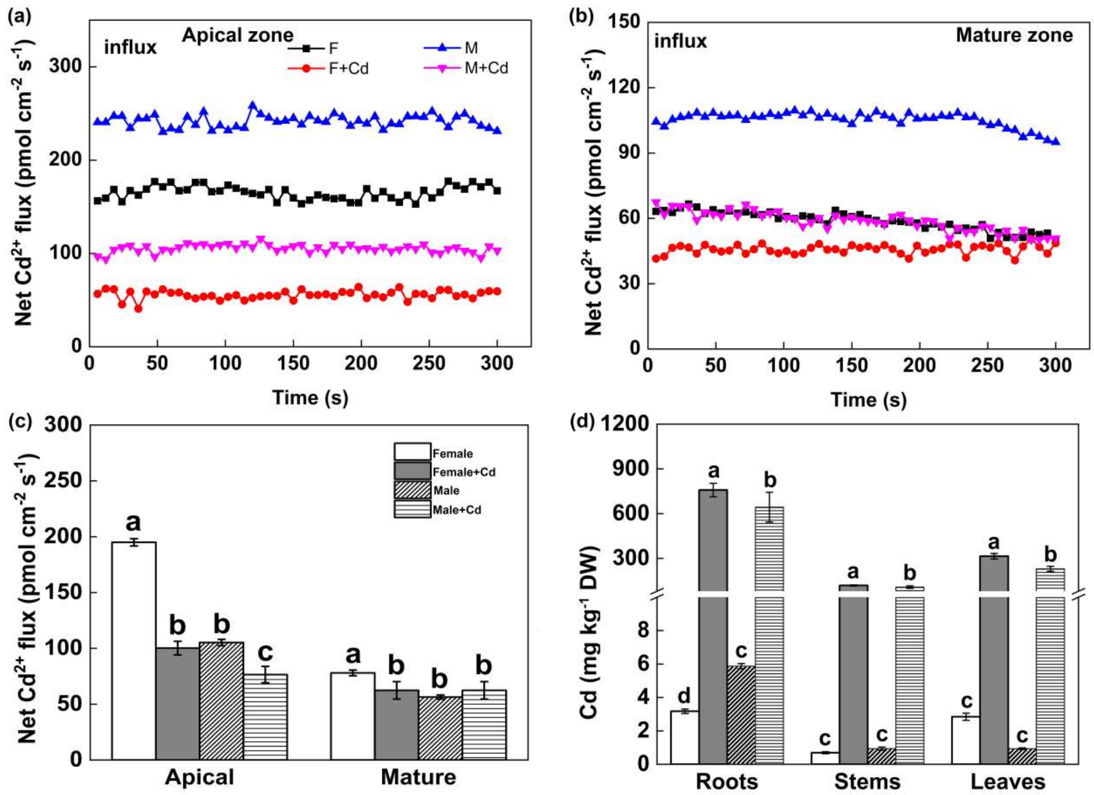
Fig. 5. Effects of Cd stress on hydrogen peroxide (H_2O_2) production (a), H_2O_2 localization (b), malondialdehyde (MDA) (c) and superoxide radical (O_2^-) production (d) in leaves and roots, and on glutathione (GSH), oxidized glutathione (GSSG) and GSH/GSSG (e), total ascorbate (TA), ascorbate (ASC) and ASC/DHA (dehydroascorbate) (f) levels in leaves of *P. cathayana* females and males. Different letters on the bars indicate significant differences between treatments. Values are expressed as means ± SD (n = 4).

Fig. 6. Hierarchical clustering of differentially expressed genes identified in the leaves and roots when only induced in females (a) or males (b) by Cd stress. FC, female-Cd; FCd, female+Cd; MC, male; MCd, males+Cd.

Fig. 7. Hierarchical clustering of key genes involved in Cd uptake, transport and detoxification in roots and leaves of *Populus cathayana* females and males exposed to Cd stress. FC, female-Cd; FCd, female+Cd; MC, male; MCd, males+Cd.

Fig. 8. A schematic model for *Populus cathayana* females and males for Cd uptake, accumulation and tolerance. Arrow thickness in leaves represents the intensity of effects. ABCC1, ATP-binding cassette transporter 1; MTP1, metal tolerance protein 1; ZIP2, zinc/iron regulated transporter related 6.2; NRAMP1, CCH1, ZIP6.2, zinc/iron regulated transporter related 6.2; HMA4, heavy metal ATPase; NRT1, nitrate reductase 1; HA2.1 AHA10.1, VHA1.1, plasma membrane; YSL2, yellow stripe-like 2; GSH, glutathione; PCS, phytochelatin synthetase family protein; ROS, reactive oxygen species; PM, plasma membrane; CW, cell wall, V, vacuole.

Figure 1



(a)
influx
Apical zone
F
M
F+Cd
M+Cd
Net Cd2+ flux (pmol cm-2 s-1)
Time (s)
(b)
influx
Mature zone
Net Cd2+ flux (pmol cm-2 s-1)
Time (s)
(c)
Female
Female+Cd
Male
Male+Cd
Net Cd2+ flux (pmol cm-2 s-1)
Apical
Mature
(d)
Cd (mg kg-1 DW)
Roots
Stems
Leaves

Figure 2

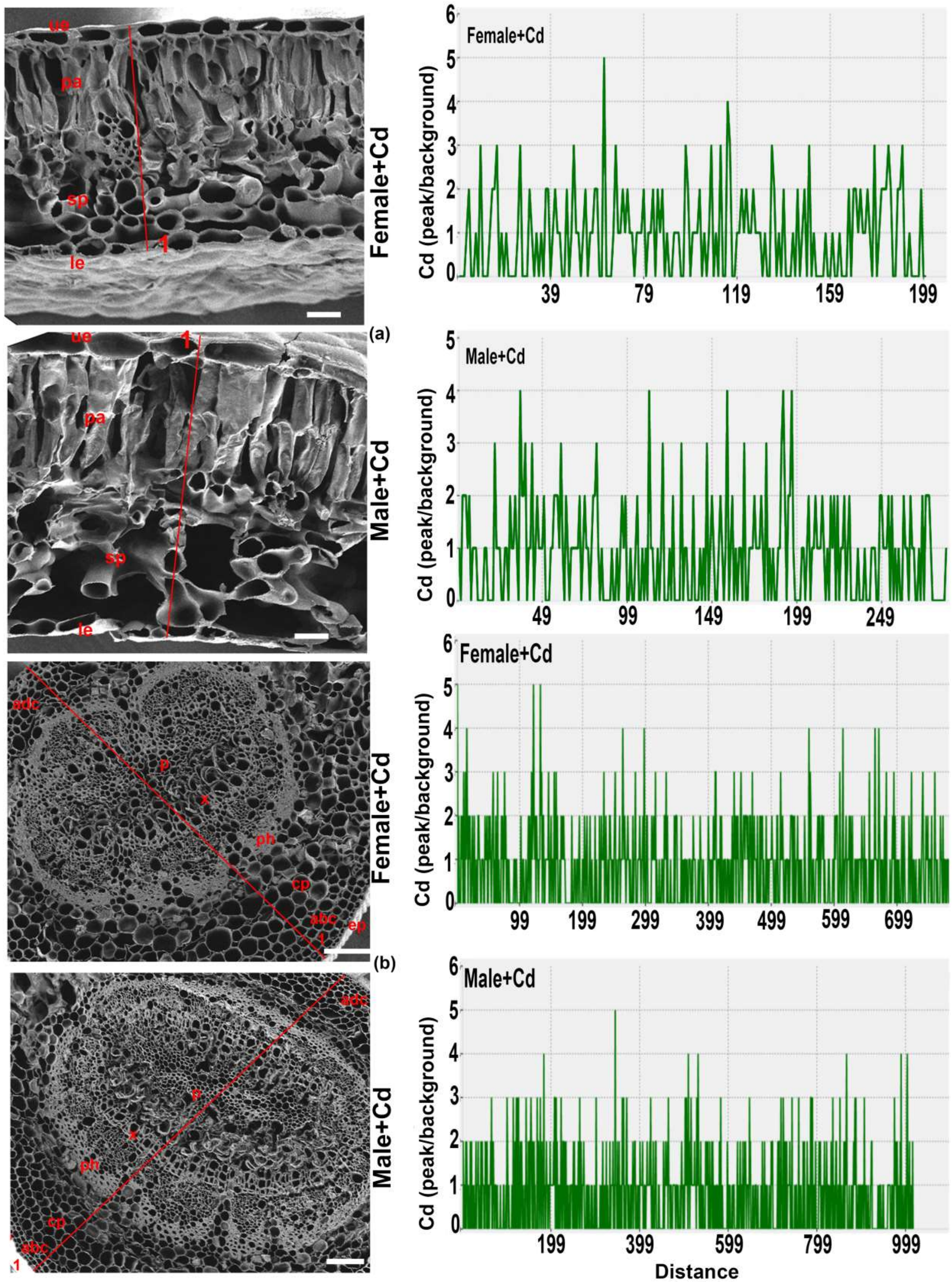
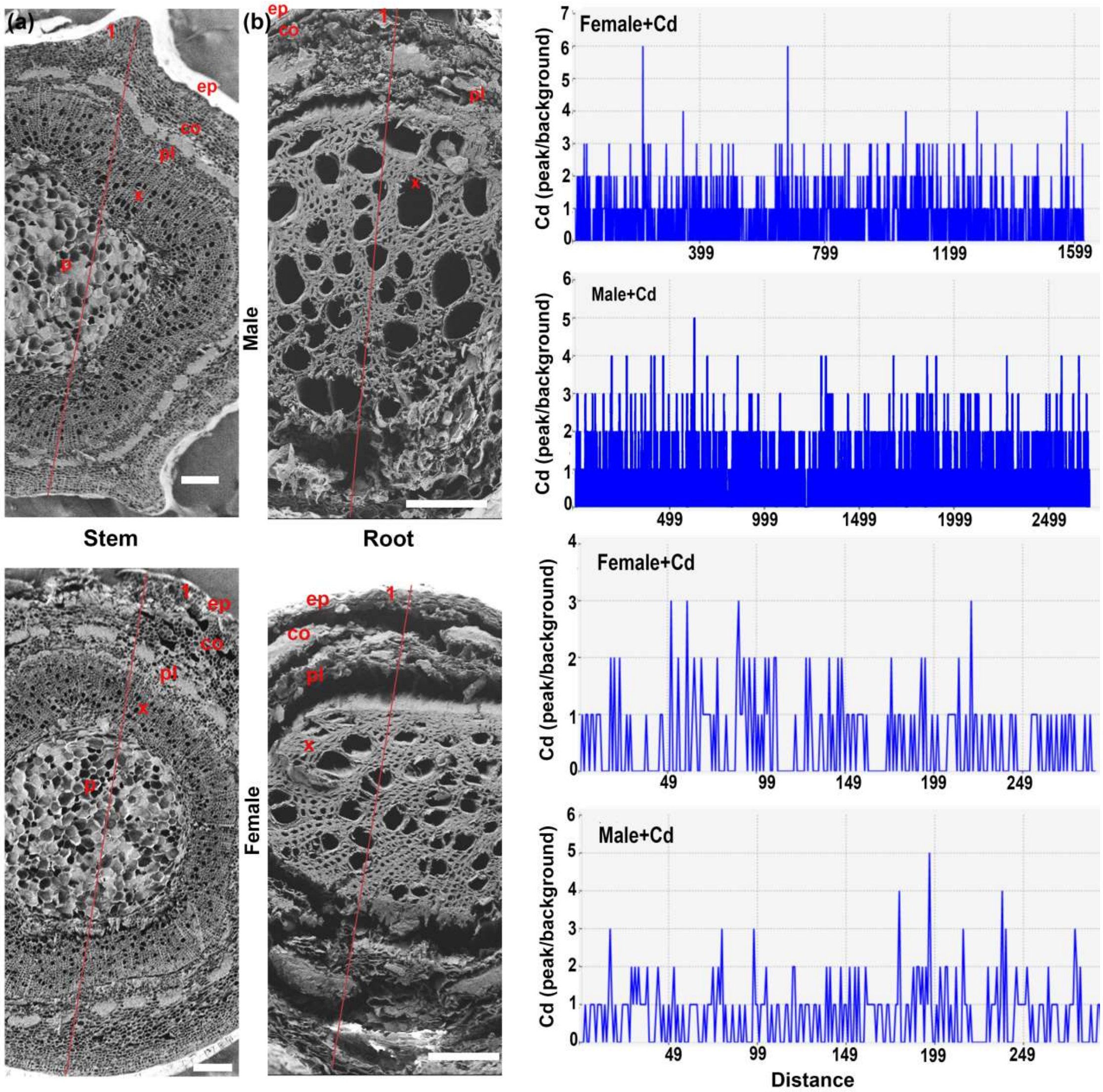

Female+Cd
Male+Cd
(a)
(b)
ue
pa
sp
le
adc
p
x
ph
cp
abc
ep
Cd (peak/background)
Distance

Figure 3



(a)
(b)
ep
co
pl
x
p
1
Male
Female
Stem
Root
Female+Cd
Male+Cd
Cd (peak/background)
Distance

Figure 4

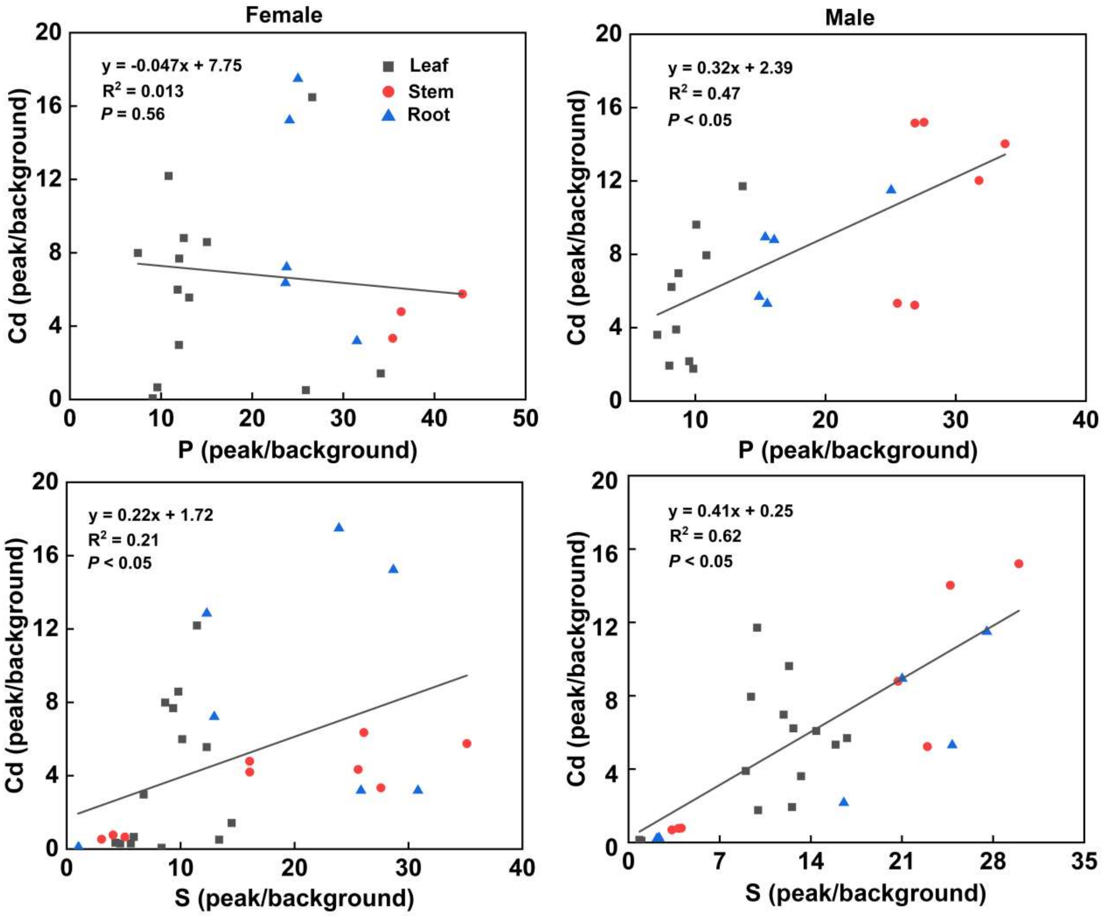

Female
Male
y = -0.047x + 7.75
R2 = 0.013
P = 0.56
Leaf
Stem
Root
y = 0.32x + 2.39
R2 = 0.47
P < 0.05
y = 0.22x + 1.72
R2 = 0.21
P < 0.05
y = 0.41x + 0.25
R2 = 0.62
P < 0.05
Cd (peak/background)
P (peak/background)
S (peak/background)

Figure 5

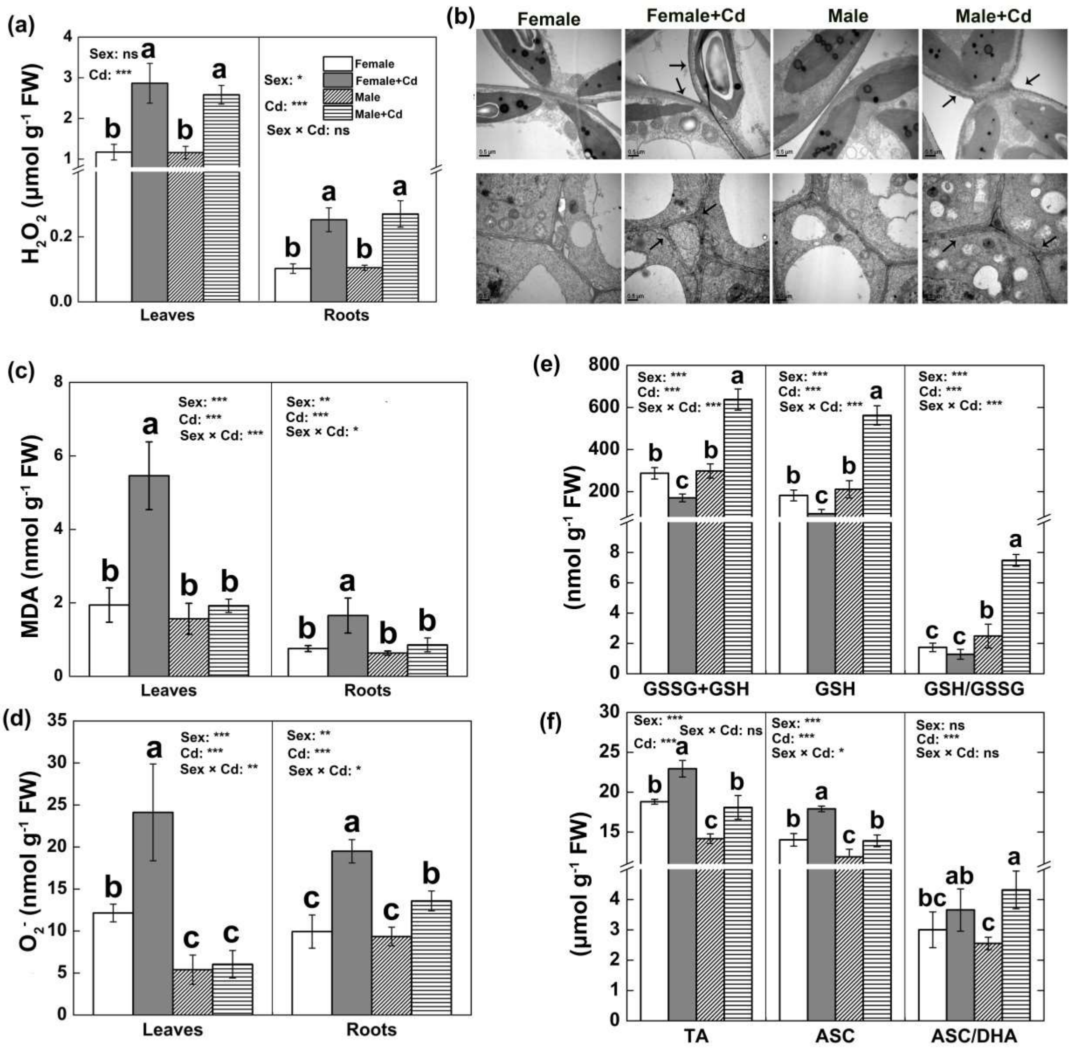
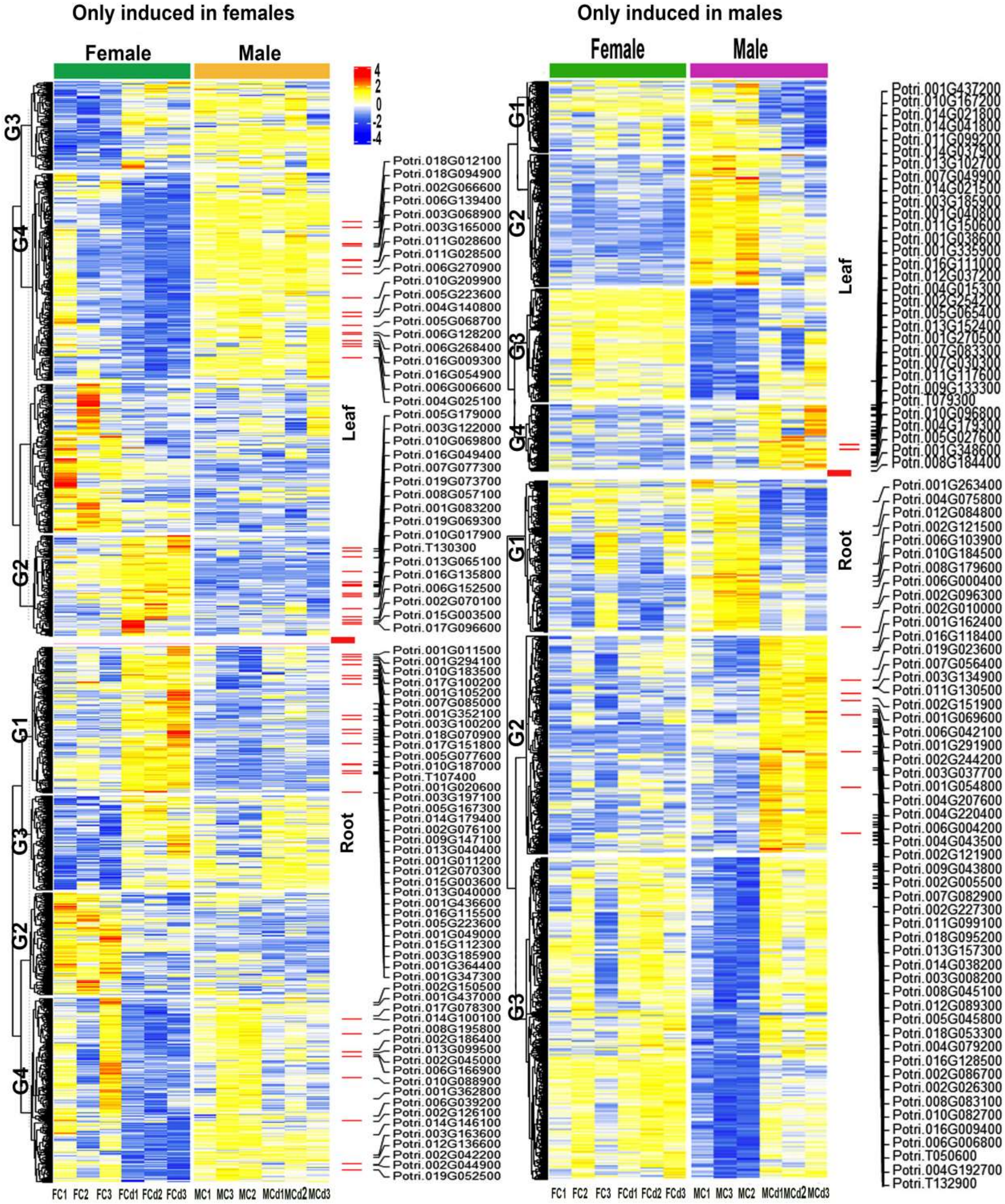

(a)
Sex: ns
Cd: ***
Sex: *
Cd: ***
Sex × Cd: ns
Female
Female+Cd
Male
Male+Cd
H_2O_2 (μmol g-1 FW)
Leaves
Roots
(b)
Female
Female+Cd
Male
Male+Cd
(c)
MDA (nmol g-1 FW)
Sex: ***
Cd: ***
Sex × Cd: ***
Sex: **
Cd: ***
Sex × Cd: *
Leaves
Roots
(d)
O_2^- (nmol g-1 FW)
Sex: ***
Cd: ***
Sex × Cd: **
Sex: **
Cd: ***
Sex × Cd: *
Leaves
Roots
(e)
(nmol g-1 FW)
Sex: ***
Cd: ***
Sex × Cd: ***
GSSG+GSH
GSH
GSH/GSSG
(f)
(μmol g-1 FW)
Sex: ***
Cd: ***
Sex × Cd: ns
Sex: ***
Cd: ***
Sex × Cd: *
Sex: ns
Cd: ***
Sex × Cd: ns
TA
ASC
ASC/DHA

Figure 6



Only induced in females
Female
Male
4
2
0
-2
-4
G3
G4
G2
G1
G3
G2
G4
Leaf
Root
Potri.018G012100
Potri.018G094900
Potri.002G066600
Potri.006G139400
Potri.003G068900
Potri.003G165000
Potri.011G028600
Potri.011G028500
Potri.006G270900
Potri.010G209900
Potri.005G223600
Potri.004G140800
Potri.005G068700
Potri.006G128200
Potri.006G268400
Potri.016G009300
Potri.016G054900
Potri.006G006600
Potri.004G025100
Potri.005G179000
Potri.003G122000
Potri.010G069800
Potri.016G049400
Potri.007G077300
Potri.019G073700
Potri.008G057100
Potri.001G083200
Potri.019G069300
Potri.010G017900
Potri.T130300
Potri.013G065100
Potri.016G135800
Potri.006G152500
Potri.002G070100
Potri.015G003500
Potri.017G096600
Potri.001G011500
Potri.001G294100
Potri.010G183500
Potri.017G100200
Potri.001G105200
Potri.007G085000
Potri.001G352100
Potri.003G100200
Potri.018G070900
Potri.017G151800
Potri.005G077600
Potri.010G187000
Potri.T107400
Potri.001G020600
Potri.003G197100
Potri.005G167300
Potri.014G179400
Potri.002G076100
Potri.009G147100
Potri.013G040400
Potri.001G011200
Potri.012G070300
Potri.015G003600
Potri.013G040000
Potri.001G436600
Potri.016G115500
Potri.005G223600
Potri.001G049000
Potri.015G112300
Potri.003G185900
Potri.001G364400
Potri.001G347300
Potri.002G150500
Potri.001G437000
Potri.017G078300
Potri.014G100100
Potri.008G195800
Potri.002G186400
Potri.013G099500
Potri.002G045000
Potri.006G166900
Potri.010G088900
Potri.001G362800
Potri.006G039200
Potri.002G126100
Potri.014G146100
Potri.003G163600
Potri.012G136600
Potri.002G042200
Potri.002G044900
Potri.019G052500
FC1 FC2 FC3 FCd1 FCd2 FCd3 MC1 MC3 MC2 MCd1 MCd2 MCd3
Only induced in males
Female
Male
G1
G2
G3
G4
G1
G2
G3
Leaf
Root
Potri.001G437200
Potri.010G167200
Potri.014G021800
Potri.014G041800
Potri.011G099200
Potri.013G102700
Potri.007G049900
Potri.014G021500
Potri.003G185900
Potri.011G150600
Potri.001G038600
Potri.001G335900
Potri.016G111000
Potri.012G037200
Potri.004G015300
Potri.005G065400
Potri.013G152400
Potri.001G270500
Potri.007G083300
Potri.007G030300
Potri.011G117600
Potri.009G133300
Potri.T079300
Potri.010G096800
Potri.004G179300
Potri.005G027600
Potri.001G348600
Potri.008G184400
Potri.001G263400
Potri.004G075800
Potri.012G084800
Potri.002G121500
Potri.006G103900
Potri.010G184500
Potri.008G179600
Potri.006G000400
Potri.002G096300
Potri.002G010000
Potri.001G162400
Potri.016G118400
Potri.019G023600
Potri.007G056400
Potri.003G134900
Potri.011G130500
Potri.002G151900
Potri.001G069600
Potri.006G042100
Potri.001G291900
Potri.002G244200
Potri.003G037700
Potri.001G054800
Potri.004G207600
Potri.004G220400
Potri.006G004200
Potri.004G043500
Potri.002G121900
Potri.009G043800
Potri.002G005500
Potri.007G082900
Potri.002G227300
Potri.011G099100
Potri.018G095200
Potri.013G157300
Potri.014G038200
Potri.003G008200
Potri.008G045100
Potri.012G089300
Potri.005G045800
Potri.018G053300
Potri.004G079200
Potri.016G128500
Potri.002G086700
Potri.002G026300
Potri.008G083100
Potri.010G082700
Potri.016G009400
Potri.006G006800
Potri.T050600
Potri.004G192700
Potri.T132900
FC1 FC2 FC3 FCd1 FCd2 FCd3 MC1 MC3 MC2 MCd1 MCd2 MCd3

Figure 7

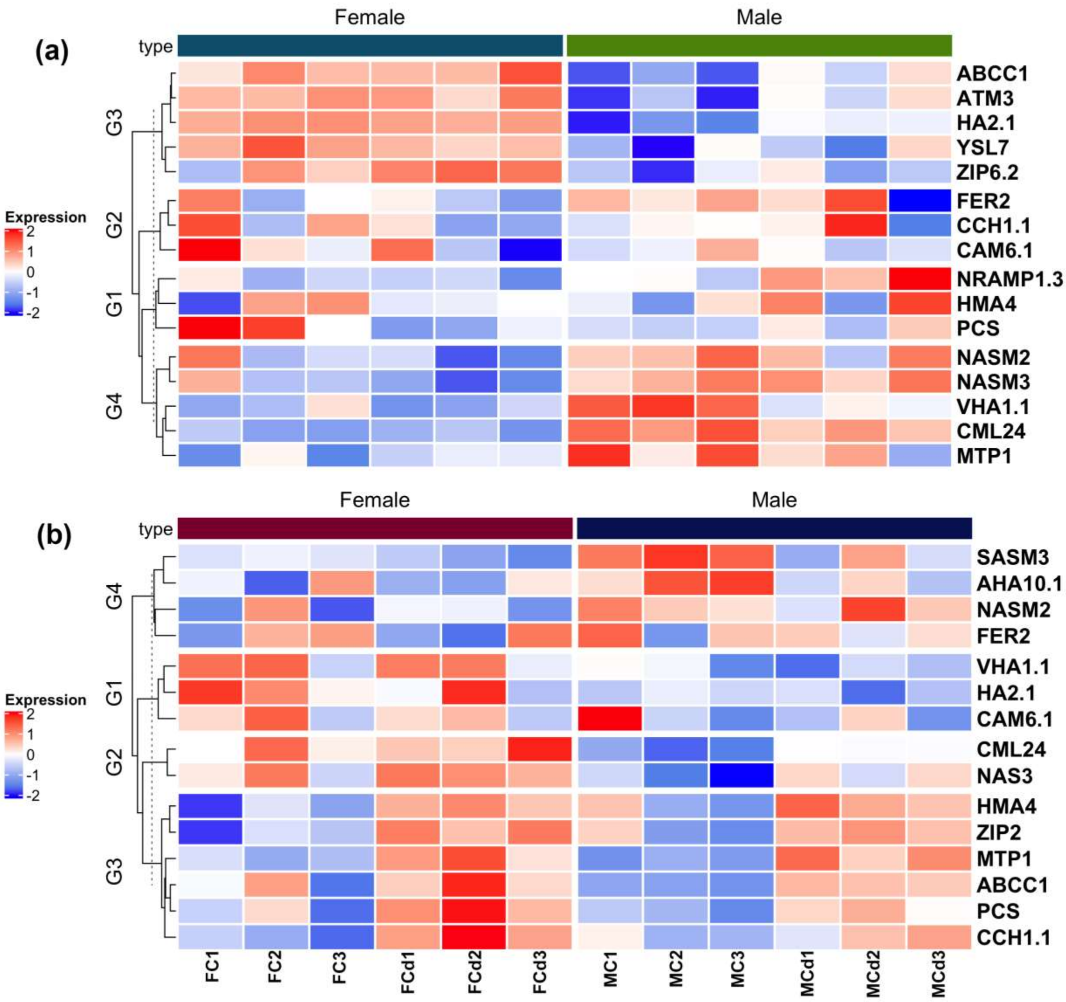
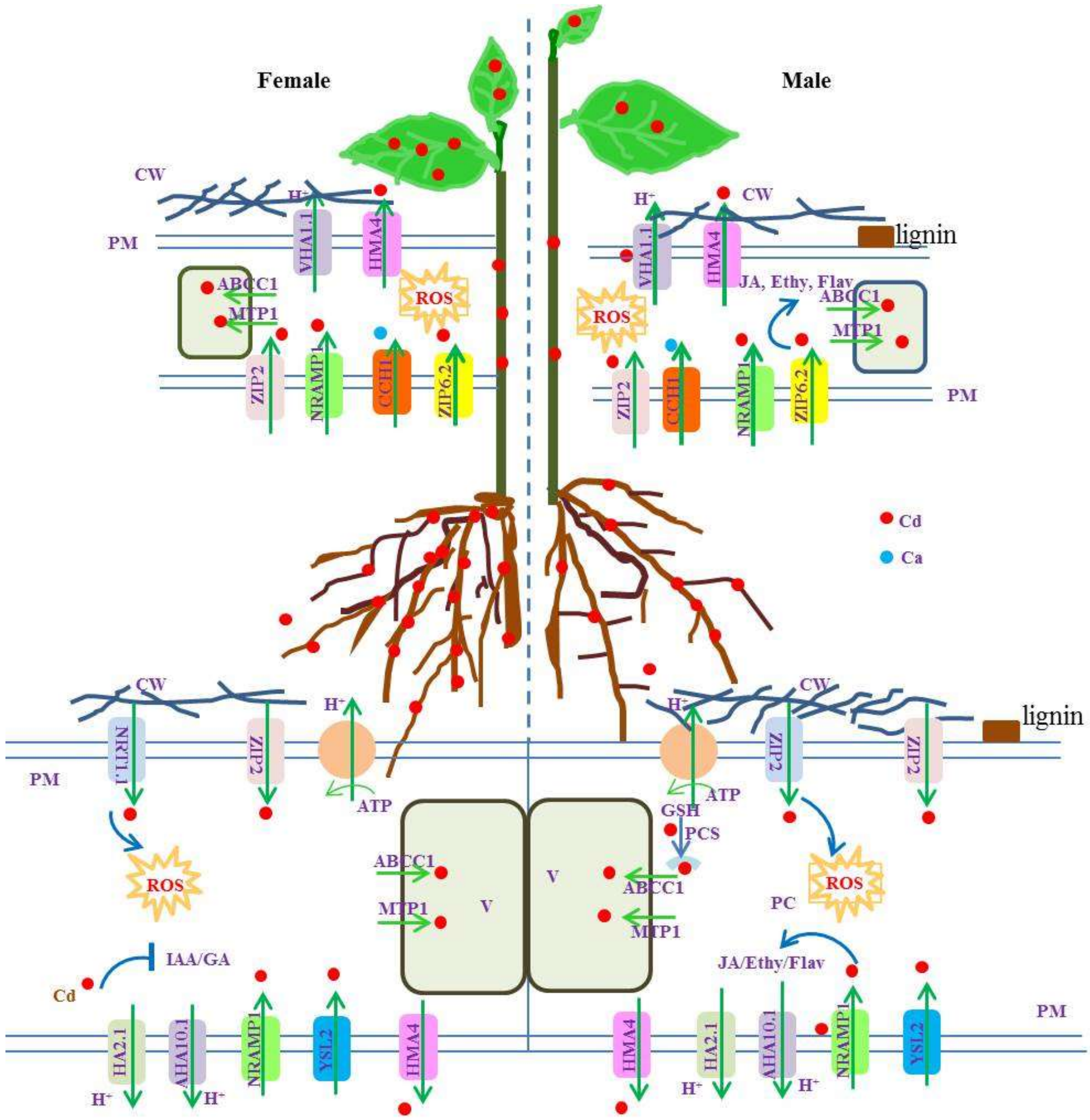

(a)
Female
Male
type
Expression
2
1
0
-1
-2
G3
G2
G1
G4
ABCC1
ATM3
HA2.1
YSL7
ZIP6.2
FER2
CCH1.1
CAM6.1
NRAMP1.3
HMA4
PCS
NASM2
NASM3
VHA1.1
CML24
MTP1
(b)
Female
Male
type
Expression
2
1
0
-1
-2
G4
G1
G2
G3
SASM3
AHA10.1
NASM2
FER2
VHA1.1
HA2.1
CAM6.1
CML24
NAS3
HMA4
ZIP2
MTP1
ABCC1
PCS
CCH1.1
FC1
FC2
FC3
FCd1
FCd2
FCd3
MC1
MC2
MC3
MCd1
MCd2
MCd3

Figure 8



Female
Male
CW
PM
VHA1.1
HMA4
ABCC1
MTP1
ROS
ZIP2
NRAMP1
CCH1
ZIP6.2
lignin
JA, Ethy, Flav
Cd
Ca
NRT1.1
ATP
GSH
PCS
V
PC
JA/Ethy/Flav
IAA/GA
HA2.1
AHA10.1
YSL2

Table 1 Net photosynthesis rate (*A*), stomatal conductance (g_s), transpiration rate (*Trm*) and stomatal limitation (L_s) in *P. cathayana* females and males as affected by cadmium stress. Different letters indicate significant differences between treatments ($P < 0.05$, Duncan's test). Control, without Cd treatment; +Cd, Cd treatment.

Sex	Treatment	*A* (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	*Trm* (mmol m^{-2} s^{-1})	L_s
Female	**Control**	16.65±0.78c	0.224±0.025b	3.96±0.49b	0.391±0.021a
	+Cd	10.97±0.57d	0.195±0.024b	3.36±0.50b	0.295±0.015b
Male	**Control**	21.24±1.50a	0.366±0.41a	5.07±0.83a	0.335±0.029b
	+Cd	19.02±0.67b	0.41±0.062a	6.07±0.70a	0.297±0.034b
P_s		***	***	***	ns
P_{Cd}		***	ns	ns	***
$P_{s \times Cd}$		**	ns	*	*

P_s, sex effect; P_{Cd}, Cd effect; $P_{s \times Cd}$, the interaction effect of sex and Cd. Different letters in the column indicate significant differences between treatments ($P < 0.05$, Duncan's test). Values are expressed as means ± SE (n = 4). The significance values of the two-way analysis of variance are shown as follows: ns, not significant; * $0.01 < P \leq 0.05$; ** $0.001 < P \leq 0.01$; *** $P \leq 0.001$.

Table 2 Fluorescence parameters *Fv/Fm*, *Y(II)*, *Y(NPQ)*, *Y(NO)*, *NPQ*, *qN*, *qP* and *ETR* in *P. cathayana* females and males as affected by cadmium stress. Different letters indicate significant differences between treatments ($P < 0.05$, Duncan's test). *Fv/Fm*, maximum quantum efficieny of PS II photochemistry; *Y(II)*, quantum yield of photochemical energy conversion in PS II; *ETR*, electron transport rate; *Y(NO)*, quantum yield of non-regulated non-photochemical energy loss in PS II; *Y(NPQ)*, quantum yield of regulated non-photochemical energy loss in PS II; *NPQ*, non-photochemical quenching parameter describing regulated dissipation of excess energy; *qP*, photochemical quenching coefficient; *qN*, non-photochemical quenching. Control, without Cd treatment; +Cd, Cd treatment.

Sex	Treatment	*Fv/Fm*	*Y(II)*	*Y(NPQ)*	*Y(NO)*	*NPQ*	*qN*	*qP*	*ETR*
Female	**Control**	0.791±0.0074a	0.369±0.036a	0.364±0.038c	0.240±0.011a	1.70±0.12b	0.709±0.030c	0.639±0.040a	42.0±3.83a
	+Cd	0.739±0.0083c	0.188±0.039c	0.588±0.041a	0.213±0.0067b	2.63±0.21a	0.837±0.017a	0.419±0.063b	19.5±2.36c
Male	**Control**	0.788±0.0052ab	0.402±0.031a	0.348±0.026c	0.257±0.0074a	1.41±0.22b	0.672±0.052c	0.655±0.033a	44.9±2.31a
	+Cd	0.776±0.01b	0.294±-0.062b	0.444±0.035b	0.244±0.021a	1.58±0.24b	0.766±0.030b	0.578±0.080a	37.6±2.56b
P_s		***	**	***	**	***	**	**	***
P_{Cd}		***	***	***	*	***	***	***	***
$P_{s \times Cd}$		***	ns	**	ns	**	ns	*	***

P_s, sex effect; P_{cd}, Cd effect; $P_{s \times Cd}$, the interaction effect of sex and Cd. Different letters in the column indicate significant difference between the treatments ($P < 0.05$, Duncan's test). Values are expressed as means ± SE (n = 4). The significance values of the two-way analysis of variance are shown as follows: ns, not significant; * $0.01 < P \leqslant 0.05$; ** $0.001 < P \leqslant 0.01$; *** $P \leqslant 0.001$.