

The multiple effects of exogenous silicon on alleviation of cadmium toxicity in Raspberry (*Rubus idaeus* L.)

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Research Article

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Abstract

Aims Cadmium (Cd) is a heavy metal, which is highly highly toxic to humans and plants. Exogenous silicon (Si) has been shown to alleviate Cd toxicity in various plant species, but the mechanism in raspberry has not been cleared.

Methods A pot experiment was adopted to evaluate the effects of Si on the growth, biochemical parameters, ultrastructure and Cd content of Cd-treated raspberry plants. In this experiment, raspberry was treated with three levels of cadmium (0, 5, and 10 mg kg⁻¹), and each level of cadmium was treated with three levels of silicon (0, 100 and 200 mg kg⁻¹).

Results The exogenous application of Si (especially at 200 mg kg⁻¹ level) obviously enhanced plant growth, photosynthetic pigments, gas exchange attributes, and ameliorated Cd-induced ultrastructural alterations by reducing malondialdehyde (MDA) content and further increasing enzymatic antioxidative defense system activity. Si applied also reduced available Cd content in the soil, resulting in lower Cd content in plant tissues and the translocation factor.

Conclusions This study revealed that external application of Si alleviates the adverse effect of Cd by enhancing the growth of plant, increasing photosynthesis, strengthening the antioxidant defense systems, reducing oxidative injury, amending Cd-induced ultrastructural changes and reducing Cd bioavailability, thereby decreasing Cd uptake and translocation with different Cd levels in raspberry.

Highlights

- Application of Si significantly alleviated Cd toxicity in raspberry.
- Supplement of Si in Cd-treated plants alleviated Cd-induced reduction in photosynthesis by further improving antioxidative systems.
- Application of Si mitigated Cd-induced ultrastructural changes and reduced Cd bioavailability.
- Addition of Si effectively reduced Cd uptake and accumulation.

1.Introduction

Heavy metal (HM) pollution is the most widespread around the world and has become a serious global problem due to the disposal of polluted sewage sludge, excessive use of chemical fertilizers, the rapid growth of industrialization and urbanization^[1]. In particularly, cadmium (Cd) is one of the most hazardous heavy metals for its high mobility, toxicity and persistence^[2], which is easily absorbed by roots of plants and then transported to above ground part. When Cd accumulation in plants exceeds their resistance values, it leads to phytotoxicity and negatively with diverse metabolic processes include growth inhibition, ultrastructural disruption, photosynthesis inhibition, reduction of essential nutrients or generation of oxidative stress^{[3][4][5]}. More importantly, Cd eventually enter the human body through the food chain, which in turn causes several diseases including osteoporosis and cancer^{[6][7]}. Therefore, it is imperative to take efficient strategies to alleviate Cd toxicity and reduce Cd uptake and accumulation in plants for food safety and human healthy^[8].

In this regard, the application of exogenous silicon(Si) has been reported to significantly alleviate various kinds of biotic and abiotic stresses^{[9][10][11]}. Being the second most abundant element in the surface of earth crust and the soil, Si is considered a beneficial element for plant growth and development^[12].Plants can take up Si from the soil in the form of silicic acid using their roots in both passive and active forms and deposit in plants^[13]. It is widely accepted that external application of Si might be a more appropriate technique to minimize or alleviate Cd entrance, translocation, and accumulation in plants as well as human dietary systems for its pollution free and non-corrosive^[14]. The effects of Si have previously been proven to be useful for increasing HMs tolerance in cotton^[15], rice^[16] and barley ^[17]. From previous research, Si-mediated mitigation of Cd stress on plants can through influencing pH and reducing the availability of toxic metal elements in soil^[18], the production of compounds, such as the number of grana, chloroplast size and chlorophyll contents^[19], elicitation of antioxidant defense to mitigate the negative effects of reactive oxygen species (ROS) in plant^[20], maintenance of membrane integrity^[21], recovery of cell ultrastructure^[22], enhance uptake and accumulation of essential nutrients^[7], reduction of heavy metal uptake and accumulation^[23], and complexation and compartmentalization of metal ions in plant tissues^[24].

Raspberry (*Rubus idaeus* L.) is a perennial deciduous shrub, and is considered as an important commercial fruit due to its typical flavor and high content of potentially important health-beneficial constituents, such as ellagitannins and anthocyanins^[25]. Studies have shown that raspberry has prevention of cardiovascular disease^[26], anti-inflammatory^[27], and antioxidant^[28] pharmacological effects and is widely planted in the world. Hence, the use of effective approach to help raspberry increase tolerance is a major concern, particularly which are cultivated in Cd-contaminated soils.

To our knowledge, there are few reports on the impact of Si for improving the tolerance of Cd stress in raspberry plants. Therefore, the present study was conducted via pot experiment to analyze the possible effects of Si on plant growth, photosynthesis, antioxidative metabolism, the cell ultrastructure changes, Cd accumulation and translocation of raspberry under Cd stress. The aim of this experiment is to provide theoretical support for relevant research and developing strategies to reduce the risks associated with Cd toxicity in raspberry plants.

2.Materials and methods

2.1 Materials

Soil samples collected from surface layer soil (0 ~ 30 cm) in Ya'an City, Sichuan Province, China. The collected soil samples were air-dried and pass through a 2 mm sieve pooled and mixed thoroughly. The major physicochemical properties of the soil were provided in Table 1.

Four-month-old raspberry (*Rubus idaeus* L.) seedlings were obtained from Aba Prefecture (Sichuan, China). Plant samples in similar growth condition were selected, and the height of plants were approximately 8 cm.

Table 1
Initial physiochemical characteristics of dry soil

Soil Characteristics	pH	Organic C (g kg ⁻¹)	Total N (g kg ⁻¹)	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)	Available Cd (mg kg ⁻¹)	Available Si (mg kg ⁻¹)	Total Cd (mg kg ⁻¹)	Total Si (mg kg ⁻¹)
Value	5.97	16.75	1.45	8.45	76.4	0.04	37.6	0.10	102.6

2.2 Experimental treatments and plant growth conditions

The pot experiment was established in a completely randomized design with two factors: three Cd treatments in 0, 5 and 10 mg kg⁻¹ which represent the actual Cd levels in some parts of our country and three Si treatments in 0, 100 and 200 mg kg⁻¹ based on the experimental design, which was performed with nine treatments: (1) control (neither Cd or Si supply), (2) Si100(100 mg kg⁻¹ Si), (3) Si200(200 mg kg⁻¹ Si), (4) Cd5(5 mg kg⁻¹ Cd), (5) Cd10(10 mg kg⁻¹ Cd), (6) Cd5 + Si100(5 mg kg⁻¹ Cd and 100 mg kg⁻¹ Si), (7) Cd5 + Si200(5 mg kg⁻¹ Cd and 200 mg kg⁻¹ Si), (8) Cd10 + Si100(10 mg kg⁻¹ Cd and 100 mg kg⁻¹ Si), (9) Cd10 + Si200(10 mg kg⁻¹ Cd and 200 mg kg⁻¹ Si). All treatments contained six replicates, three of which were used for growth, gas exchange attributes and Cd content measurement, and the other three used for pigment assay, malondialdehyde, antioxidant enzyme activities and ultrastructure observation. The 10 kg of dried soil was loaded into each plastic pot (30 cm diameter×26 cm high). Cd (in the form of CdCl₂·2.5H₂O solution) was artificially added to make total Cd of the soil at the required amount. The contaminated soil kept for regularly weathering for 3 months under both wet and dry condition to stabilize (leaching was avoid). Two weeks before transplanting, Si (in the form of Na₂SiO₃·9H₂O solution) was added according to the experimental design. To compensate the additional of Na caused by Na₂SiO₃·9H₂O application, equivalent NaCl was added according to the highest concentration of Si treatment and the final pH of the solution was maintained 6.5 using NaOH and HCl. Soil samples of all treatments were taken before transplanting to determine soil available Cd concentration.

In May 2020, the uniform-sized seedlings were transplanted, which were then randomly placed inside an isolation chamber with natural sunlight and temperature. The whole pot experiment was carried out at the research area of Wen Jiang campus, Sichuan Agricultural University in Chengdu City, China (103°51'29"E, 30°42'18"N). During experimental treatments, seedlings were regularly watered to maintain at 70% of the soil water holding capacity, and NPK fertilizer was applied at a rate of 5 g kg⁻¹ to each pot monthly to avoid nutrient deficiencies. After 100 days of transplantation, all plants were harvested for further assessment. The fresh matured leaves and roots were immediately frozen in liquid nitrogen and then stored in a refrigerator at -80°C to measure physiological parameters.

2.3 Measurement of plant growth

The harvested plants were divided into leaves, stems and roots. The fresh tissues were soaked in 10 mmol L⁻¹ Na₂EDTA for 30 min to remove the Cd ions that adsorbed on the surface and then thoroughly washed with deionized water. The root length was determined. Then the samples were kept in a hot air oven at 105°C for 3 h and at 75°C to reach a constant weight and assessed the dry weight (DW) of the leaves, stems, roots and total.

These dried samples were ground to fine power for Si and Cd contents analysis at last. Meanwhile, the soil samples were uniformly composited and air-dried to measure the physical and chemical properties.

2.4 Measurement of gas exchange attributes and photosynthetic pigments

Gas exchange attributes including net photosynthesis rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and transpiration rate (T_r) of the second mature leaf from the top of the canopy were measured from 9:00 am to 11:00 am on a sunny day using the portable photosynthesis system (LI-6800, LI-COR Inc., USA) after 100 days of Cd & Si treatment.

The photosynthetic pigments of fresh leaves were determined by homogenizing 0.5 g leaf tissue with 10 ml chilled 80% acetone and centrifuged (5000×g, 5 min, 4°C). The extracts were determined at 470, 646 and 663 nm. The chlorophyll (a, b), total chlorophyll and carotenoid content were calculated according to Lichtenthaler and Wellburn^[29].

2.5 Measurement of malondialdehyde (MDA) and antioxidant enzyme Activities

About 0.5g weighted frozen raspberry leaf and root materials were ground to powder in liquid nitrogen and extracted in (50 mM) phosphate buffer (PBS, pH 7.8) to determine the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). The homogenates were centrifuged at 12,000 ×g for 15 min and stored at 4°C. Antioxidant enzyme activities were assessed using the kits of Nanjing Jiancheng Bioengineering Institute (<http://www.njjcbio.com>) according to Mapodzeke^[30].

Malondialdehyde (MDA, a product of lipid peroxidation) content was assessed through 2-thiobarbituric acid (TBA) reactive metabolites according to the modified method of Chen et al.^[7]. Briefly, 0.2 g weighted fresh samples were homogenized and extracted in 5 ml of 0.25% TBA made in 10% trichloroacetic acid (TCA) and centrifuged at 10,000 ×g for 20 min at 4°C. The supernatant was measured for MDA content at 600, 532, and 450 nm.

2.6 Examination of leaf and root ultrastructure

Small sections (1 mm²) from the middle of the upper second fully expanded leaves and root tips (2 ~ 3 mm) collected at 5 cm from the apex were prefixed with a 3% glutaraldehyde in 0.1 mM phosphate buffer (pH 7.2) for 6–8 h, post-fixed in 1% OsO_4 (pH 7.2), and then thoroughly washed three times with the same buffer. Subsequently, the samples were dehydrated in an acetone series (30, 50, 70, 90, 95, and 100%) and embedded in epoxy resin overnight. An ultrathin section was performed using Ultracut E ultramicrotome (Leica, Germany). Samples were stained with aqueous uranyl acetate followed by lead citrate and finally examined under TEM JEM 1400 (JEOL Ltd., Japan) at 80.0 kV. The images that best represented the changes in the ultrastructure were selected.

2.7 Measurement of Cd in plant tissues and soil available Cd

Dried raspberry plants samples (leaves and stems 0.2 ± 0.0005 g and roots 0.1 ± 0.0005 g) were digested with 10 ml of mixed acid (HNO_3 : $\text{HClO}_4 = 9:1$, v/v), and an extraction solution of Ammonium bi-carbonate di-amine tri-ethylene penta-acetic acid (AB-DTPA) was prepared to determine the bioavailable Cd in soil^[31]. Inductively coupled plasma mass spectrometry (ICP-MS, Nexion300x, PerkinElmer, Inc., USA) was used to determine Cd concentration.

For quality assurance, GBW07603 (GSV-2; bush twigs and leaves) and GBW07428 (GSS-14; Sichuan basin soil) from the center for the standard reference of China were analyzed, too.

The translocation factors (TFs) were calculated to evaluate the efficiency of phytoextraction. The TF values for Cd are defined as:

TF = the concentration of Cd (mg kg⁻¹) in plant shoot/the concentration of Cd (mg kg⁻¹) in plant root.

2.8 Statistical analysis

Statistical analysis was performed using the SPSS v. 22.0(SPSS Inc., Chicago, IL, USA) as mean of at least three replicates ± standard deviation (SD). Two-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range (DMR) test to determine the significant differences (at the P < 0.05) between treatments. Graphs were illustrated using Sigmaplot v.14.0 (Systat Software Inc., USA) and Microsoft PowerPoint v.2019.

3 Results

3.1 Plant growth

Compared with control, the dry weight of leaves, stems, roots, total and root length significantly decreased under two Cd levels (Cd5 and Cd10), and reduction was more pronounced when the higher level of Cd was applied (Table 2). In addition, Si applied alone without Cd had no significant effects over the control. However, the application of Si100 and Si200 all alleviated the negative influence of the Cd treatments, and Si200 was better than Si100 to alleviate the inhibition of Cd on the growth of raspberry plants.

Table 2
Effects of Si on growth in raspberry plants under Cd stress.

Treatments	Leaf Dw (g plant ⁻¹)	Stem Dw (g plant ⁻¹)	Root Dw (g plant ⁻¹)	Total Dw (g plant ⁻¹)	Root length (g plant ⁻¹)
CK	66.38 ± 2.97 ab	90.54 ± 1.00 a	23.16 ± 1.49 ab	180.07 ± 5.19 a	24.74 ± 0.95 a
Si100	67.69 ± 2.78 a	91.20 ± 2.14 a	23.30 ± 0.74 ab	182.19 ± 5.34 a	25.03 ± 1.47 a
Si200	68.42 ± 2.75 a	92.02 ± 1.78 a	24.06 ± 0.66 a	184.50 ± 5.04 a	25.23 ± 0.80 a
Cd5	56.79 ± 1.08de	78.49 ± 1.83 d	18.18 ± 0.76 de	153.46 ± 3.63 d	20.74 ± 0.48 d
Cd5 + Si100	60.81 ± 1.75 c	83.57 ± 2.90 bc	19.88 ± 1.09 cd	164.26 ± 2.56 bc	23.01 ± 0.66 bc
Cd5 + Si200	62.79 ± 1.80 bc	86.27 ± 0.66 b	21.65 ± 1.32 bc	170.71 ± 3.48 b	23.99 ± 1.43 ab
Cd10	41.47 ± 1.96 f	55.98 ± 4.04 f	15.11 ± 0.93 f	112.55 ± 5.79 f	16.75 ± 0.58 f
Cd10 + Si100	55.30 ± 1.89 e	72.95 ± 2.60 de	17.05 ± 1.47 e	145.30 ± 1.56 e	19.09 ± 0.54 e
Cd10 + Si200	59.65 ± 1.87 cd	80.67 ± 2.69 cd	19.09 ± 1.05 d	159.41 ± 1.82 cd	21.55 ± 0.70 cd

Means ± SD (n = 3) with different letters in the same column indicate significant difference by Duncan's multiple range test (P < 0.05) at different treatments.

3.2 Photosynthetic pigments

The effects of Si treatment on the content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids value in raspberry leaves under Cd stress are assessed in Fig. 1. The recorded data demonstrated that Si100 and Si200 did not result in significantly different of raspberry pigments in the absence of Cd stress. No surprisingly, increasing Cd levels remarkably decreased the chlorophyll and carotenoids in treated raspberry plants than that of control. After the addition of Si (both Si100 and Si200) at two Cd levels (Cd5 and Cd10) the negative effect was alleviated. More importantly, the plants treated with Si200 showed obviously increase compared with Si100 in combination with Cd5 and Cd10.

3.3 Gas exchange attributes

The gas exchange attributes were analyzed to value the effect of Si on the photosynthetic capacity of raspberry under Cd stress. The P_n (Fig. 2a), G_s (Fig. 2b) and T_r (Fig. 2d) of raspberry plants were inhibited with the increasing levels of Cd as parallel to control. While Si addition mitigated these adverse effects and the effect of Si200 were the most obvious. Moreover, the increased C_i (Fig. 2c) when exposed elevated Cd levels indicated that the limiting photosynthesis of raspberry leaves was the non-stomatal restriction. Similarly, no differences were observed between control and the addition of Si alone (Si100 and Si200).

3.4 Antioxidant enzymes activities and lipid peroxidation (MDA)

Raspberry plants treated with increasing Cd levels considerable increased the antioxidant enzymes activity of SOD (Fig. 3a and b), POD (Fig. 3c and d) and CAT (Fig. 3e and f) in leaf and root tissues over the control which indicated that raspberry plants enhanced antioxidant enzymes activity to reduce oxidative damage caused by Cd stress. Exogenously applied Si additionally further stimulated the activities of all these enzymes in the presence of Cd. At the same time, application of Si was more effective at a higher rate (Si200) than at a lower rate (Si100). In the absence of Cd, the addition of Si did not affect activities of antioxidant enzymes in the leaf and root tissues as parallel to control.

All levels of Cd induced the reactive oxygen species (ROS) aggregation, which led to a considerable enhancement in MDA content. Generally, MDA concentration improved significantly with the increasing concentration of Cd than those of the control (Fig. 3g and h). In addition, there was no significant change in the separate application of Si without Cd applied as parallel to control. The recorded data showed that the MDA concentration clearly decreased in Cd treatments with both Si concentrations supply, but the Si200 application was predominant to minimize Cd toxicity. These results suggest that Si has a protective effect against Cd-induced ROS stress.

3.5 TEM observation

The mesophyll cells in the control conditions showed typical ultrastructural characteristics with elliptical chloroplasts close to the cell wall, and there were only fewer starch grains and osmiophilic granules in the chloroplasts. The grana and thylakoid membrane systems were closely arranged and stacked in an orderly manner (Fig. 4a). Compared with the control, the chloroplasts were more clear and intact under the Si100 and Si200 treatments. The cytoplasm matrix seems deeper-colored, and either the quantities or the volumes of starch grains and osmiophilic granules decreased obviously (Fig. 4b and c). With the increasing contamination of Cd, the chloroplasts became swollen and even deformed. Under Cd5 conditions, the chloroplasts were characterized by compact thylakoids squeezed by the big starch grains and by a few of bigger osmiophilic granules, while there were a large number of osmiophilic granules in chloroplasts observed under Cd10 conditions (Fig. 4d and g). Moreover, under Cd10 conditions, the nuclear membrane was broken and obscure when compared to that under control conditions (Fig. 4g). However, application of Si alleviated Cd-induced ultrastructural impairment, as indicated by

the decreased number or volume of the starch grains and osmiophilic granules, and the restoration of thylakoid membrane integrity. It seems that the mitigation effect from Si 200 are more evident because there was almost no osmiophilic granules, and ultrastructure of mesophyll cells was similar to that under control conditions (Fig. 4e, f, h and i).

The root tips cells in the control exhibited integral ultrastructure with thick cell wall and a large central vacuole (Fig. 5a), and occasionally, we observed a few round mitochondria with clear cristae and dense matrix. There was no visible alteration in ultrastructure induced by Si applied alone (Si100 and Si200) when compared with the control conditions, except for high electron-density substances deposited in the cell wall (Fig. 5b and c). However, ultrastructure of root cells was injured reflected by the swollen mitochondria and the disappeared cristae under Cd5, whereas shape of the cells seems distorted and a more serious degradation of the membrane structure occurred (Fig. 5d and g) under Cd10. A large amount of electron dense material deposited in cell wall or in the cellular membrane. In contrast, application with Si100 or Si200 altered the disruption of ultrastructure induced by Cd exposure, and more electron dense materials accumulated in the cell wall (Fig. 5e, f, h, i). Obviously, under Cd polluted conditions, Si200 induced a more pronounced alleviation effects on the ultrastructural integrity than Si100.

3.6 Cd concentration and translocation factor

No difference was observed between control and separate addition of Si (Si100 and Si200) in Cd concentration of raspberry leaf, stem and root (Table 3). Cd concentrations in all tissues increased with the increasing Cd levels in the order of roots stems leaves, and with nearly 80% of the accumulated Cd found in the roots. However, the Cd content in raspberry was significantly dropped by Si applied at two levels of Cd treated, and it is more pronounced at the application rate of Si200 than the Si100. Moreover, the Cd translocation factors (TF) of raspberry from root to shoot in this experiment were also restricted with Si applied (Table 3). Importantly, the soil available Cd contents, as compared with both Cd alone applied were slightly decreased under Si100 application and this decrease was significant at higher treatment of Si200 (Table 3).

Table.3 Effects of Si on Cd concentration and translocation factor (TF) in raspberry and soil available Cd under Cd stress.

Treatments	leaf Cd Conc. (mg kg ⁻¹ DW)	steam Cd Conc. (mg kg ⁻¹ DW)	Root Cd Conc. (mg kg ⁻¹ DW)	TF	Soil Available Cd Conc.(mg kg ⁻¹)
CK	0.017 ± 0.003 f	0.090 ± 0.011 e	0.531 ± 0.048 g		0.088 ± 0.014 e
Si100	0.018 ± 0.002 f	0.093 ± 0.004 e	0.541 ± 0.015g		0.086 ± 0.020 e
Si200	0.017 ± 0.001 f	0.096 ± 0.004 e	0.544 ± 0.013 g		0.082 ± 0.007 e
Cd5	0.251 ± 0.015 c	1.737 ± 0.065 b	9.612 ± 0.672 c	0.207 ± 0.008 a	0.765 ± 0.204 c
Cd5 + Si100	0.208 ± 0.013 d	1.276 ± 0.050 c	7.525 ± 0.360 e	0.197 ± 0.003 ab	0.744 ± 0.097 cd
Cd5 + Si200	0.174 ± 0.005 e	0.886 ± 0.046 d	5.651 ± 0.213 f	0.188 ± 0.011 b	0.580 ± 0.061 d
Cd10	0.446 ± 0.033 a	3.656 ± 0.183 a	19.649 ± 0.528 a	0.209 ± 0.006 a	1.239 ± 0.040 a
Cd10 + Si100	0.322 ± 0.016 b	1.768 ± 0.039 b	10.487 ± 0.172 b	0.199 ± 0.003 ab	1.186 ± 0.160 ab
Cd10 + Si200	0.237 ± 0.005 c	1.373 ± 0.026 c	8.507 ± 0.278 d	0.189 ± 0.003 b	1.030 ± 0.070 b

Means ± SD (n = 3) with different letters in the same column indicate significant difference by Duncan's multiple range test (P < 0.05) at different treatments.

4 Discussion

The soil contaminated with Cd increases the risk of plant growth and it is crucial to minimization of Cd uptake and accumulation to decrease Cd toxicity in plants. Although Si is not an essential nutrient for plant growth, its exogenous application is widely acknowledged as a vital source for ameliorating various stresses in plants. Our current studies demonstrated that the inhibition of raspberry growth was significantly alleviated with Si application under both Cd levels, as reflected by the photosynthetic pigments in leaves, gas exchange attributes, the ultrastructure of leaf and root cells, and the accumulation and transfer of Cd in the plant, particularly when the Si at the concentration of 200 mg kg⁻¹. Similar mitigating effects have been proved in numerous plant species^{[32][33][34][35]}.

4.1 Growth attributes

Plant growth parameters is generally considered as one of the most important factors for estimating plant resistance or/and tolerance to stress. Our present study illustrated that both levels of Cd (Cd 5 and Cd 10) reduced the growth as shown with all measured growth parameters (Fig. 1). The limitations of plant growth can be attributed as toxic effects of Cd that cause severe disorders in plant physiological and metabolic processes, such as photosynthesis, photosynthetic pigment content and absorption of essential nutrients^{[36][37][38]}. Excessive Cd concentration in plant tissues accelerated ROS generation (Fig. 3), which in turn altered ultrastructural of leaf and root cells (Figs. 4 and 5). Addition of Si have been demonstrated to alleviate the inhibitory effect of Cd on the plant growth in maize^[39] and cotton^[40]. Our study was consistent with the previous reports, but was strongly dependent

upon the concentrations. The application of Si has also been shown to promote nutrient uptake in cowpea plants, which may be one of the reasons that Si mitigates the negative effect under Cd stress^[32]. Khan et al.^[20] agreed that the application of Si could enhance the growth of date palm under Cd stress, and this can be attributed to the reduction of Cd accumulation in date palm through augmenting plant growth and biomass by Si application that can lead to the inhibition of Cd uptake and transport. In addition, Si can regulate gene expression, sequester Cd in the root cell walls and organelles, and reducing Cd transfer to the shoots^[41].

4.2 Photosynthetic physiological attributes

Cd stress would change pigment contents in plant leaves, thereby changing the photosynthetic capability of plant leaves^[42]. The present results reported that chlorophyll a, chlorophyll b, total chlorophyll and carotenoids contents were significantly inhibited in treatment Cd5 and further reduced in Cd10 (Fig. 2), similar to in maize^[34]. The possible reason of Cd-induced the decreasing photosynthetic pigments contents in the raspberry plants could be due to elevation in Cd-induced oxidative stress and stimulation of chlorophyllase enzyme, which impairs integrity chloroplast structures and chlorophyll synthesis^{[22][43]}. Additionally, study have shown that Fe deficiency, which is a common response to Cd or other heavy metal stress, also is one of reason limits chlorophyll synthesis^[4]. Undeniably, application Si under Cd stress, the increase of photosynthetic pigments of raspberry plants in our study can be attributed to decrease in lipid peroxidation and further increase in the activities of antioxidant enzymes (SOD, POD, and CAT) in our study, which is accordance with previous study^[44].

Based on our study, the values of P_n , G_s , and T_r were significantly decreased while C_i increased obviously which indicated that the main reason for decreasing the photosynthesis of raspberry was primarily non-stomatal limitations under Cd stress. In the presence of Cd, the increase in C_i may be due to the alterations in chloroplast ultrastructure and the inhibition of pigment synthesis, which affects carbon fixation^[45]. The addition of Si suppressed the negative effect under Cd stress by boosting up P_n , G_s , and T_r , whereas declining C_i , implying that Si application enhanced raspberry plants resistance by decreasing non-stomatal limitation, promoting chloroplast ultrastructure integrity and Calvin cycle enzyme activity. Feng et al.^[46] found that application Si alleviated the negative effect caused by Cd through significantly increasing G_s , while reducing C_i of *Cucumis sativus* under Cd stress.

4.3 Antioxidant defense mechanism and lipid peroxidation (MDA)

Plants can synthesize enzymatic antioxidants to eliminate excess ROS, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT)^[47]. Our study illustrated that both levels of Cd obviously boosted up antioxidant enzymes contents including SOD, POD and CAT in raspberry plants and Si application further prompted in all enzyme activities. It is played a vital role that these raised enzymatic activities were progressed and reached their maximum by exogenously application of Si to mitigate the adverse effects of Cd-induced oxidative damage. Parallel impacts of Si on stimulation of antioxidant systems under Cd toxicity have been previously reported in wheat^[33] and pepper^[48]. Thence, Si might alleviate oxidative stress induced by Cd, at the cellular level, due to more efficient use of metabolic pathways that scavenge ROS, which may protect the integrity of cell membranes. Furthermore, it is noteworthy that the enhancement in antioxidant system seemed to be better in plants treated with Si200 than Si100.

Malondialdehyde (MDA), as an end product of lipid peroxidation, can reflect the degree of stress/membrane damage induced by Cd^[49]. Based on the obtained data, the inhibition in plant growth by Cd was linked with Cd-induced MDA accumulation, which indicates that Cd may cause oxidative damage to plants^[21]. Whereas, exogenous usage of Si considerably lowered the amount of MDA in raspberry leaves over the respective control plants, and a higher reduction ratio was observed in Si200. These data suggest Si application may be result in the recovery of cell membrane injury and the maintenance of cell membrane integrity in raspberry plants^[50]. Our results are supported by xiao et al.^[16] who reported that silicon fertilizer promotes antioxidant defense response against Cd-induced oxidative stress in rice. Gheshlaghpour et al.^[51] reported that Si treatment assists in retaining the integrity, stability, and activity of plant cell membrane by reducing membrane permeability and lipid peroxidation. The presence of Si in plant growing medium decreases Cd uptake through root and then decreases the transfer of Cd to shoot which reduces Cd-induced cellular damages^[52].

4.4 TEM observation

TEM is a useful tool to evaluate cellular integrity and previous studies have shown Cd-caused ultrastructural modification and disorder in mesophyll cells or root tips cells^{[30][53][54]}. In our study, the ultrastructural changes in the mesophyll cells induced by Cd seem to be dose-dependent. With the increase of Cd pollution, impairments in ultrastructures of mesophyll cells became more serious, as shown by the increase in the number and size of starch grains and osmiophilic granules, which are similar to the observation in *Oryza sativa* L.^[55]. Moreover, the starch accumulation in chloroplasts might contribute to the reduction of photosynthetic capacity in raspberry when exposed to Cd exposure, because excessive accumulation of starch in chloroplasts usually results in photosynthetic inhibition because of negative feedback regulation (Chen et al., 2014)^[56]. Fundamentally, the massive accumulation of starch granules generally results from nutrient deficiency, decrease of sink force or disturbance of sugar loading systems in veins under Cd stress^{[4][40]}, indicating the possible negative effects exerted from Cd exposure. In addition, the great increase in the number of osmiophilic granules, as a intermediate product in thylakoid metabolism, suggested that inner membrane degradation in the chloroplasts^[57]. Interestingly, we found that application of Si mitigated injury extent in chloroplasts induced by Cd contamination, particularly when applied with Si200. In line with our results, Vaculík et al.^[34] suggested that Si can enhance biomass production by improvement of thylakoid formation and thus increase efficiency of photosynthesis in maize.

We noticed that the ultrastructure of root tips cells showed visible impairments under Cd stress, and application with Si can protect cellular integrity. Such alleviation effects of Si probably be correlated with redistribution of the electron dense materials, as shown by that Si treatment enable the cell wall to accumulate more electron dense materials. Such electron dense deposition material scattered in the cell wall also was observed in *Brassica napus* L.^[58]. Because Cd retention in the cell walls is one of the most important mechanisms to lessen Cd toxic effects on protoplasts in plant cells^{[38][59][60][2]}, it is reasonable to think that Si-induced reduction of electron dense materials in cytoplasm contributes to the mitigation of Cd toxicity.

4.5 Effects of Si on the Cd content

Increased heavy metals concentration inside the plant tissues can negatively affect its growth and developmental processes. Much more Cd was tested in both leaves, stems and roots with Cd concentration increased, and mainly accumulated in roots rather than in the aerial parts implying that plants do not translocate all cadmium absorbed by the plant to the above-ground biomass. Importantly, significant effects were detected that exogenous of Si reduced Cd concentration in raspberry plants, particularly when the plants were treated with Si200 under Cd stress.

This is in accordance with the results of Kollarova et al.^[61], who observed maize protoplasts of both hybrids decreased in Cd uptake in the presence of Si. The reduced Cd levels in plants in the presence of Si can be attributed in many ways such as by increasing nutrient uptake in plants which may inhibit the Cd accumulation^[44], by enhancing oxalate exudation of roots to decrease Cd content^[62] and by up-regulating the expression of the Cd efflux-related gene^[63]. Our study indicated that reducing the MDA production (Table. 3) and keeping the cell ultrastructure in plants (Fig. 4&Fig. 5) that alter the photosynthesis (Fig. 2) and accelerate the growth (Fig. 1) to achieve a dilution effect^[5]. As we also determined that the application of Si decreased the AB-DTPA Cd (Fig. 6), so another mechanism for reducing Cd concentration may be due to the co-precipitation and immobilization of metal ions by Si, hence reducing available Cd concentration and Cd influx^[50].

The observation of translocation factor is an essential way to track the uptake of heavy metals from root to shoot as it reflects plant ability to increase or reduce metal transport^[30]. Our study highlighted that Silicon addition also declined the transfer factors (TF) of Cd from root to shoot. This was similar with the relationship between Si supply and Cd stress in wheat^[23]. Additionally, the TF for raspberry under all the Cd treatments were found to be < 1 in our study, which indicated that Cd accumulation in roots as a tolerance mechanism of the tested plant species to alleviate disturbances in various physiological and biochemical processes^[64]. Our experimental results showed that all Si applied resulted in a significant decrease in soil available Cd (Table 1), leading to the increased growth and reduced Cd accumulation (Figs. 1 and 2). This effect responds positively to the application of Si to reduces soil cadmium bioavailability and promote health wheat growth and yield^[65]. The application of Si may reduce the metal bioavailable fraction in the soil by improving the soil pH, thereby affecting the metal speciation in the soil and decreasing Cd availability^[66].

5 Conclusion

In conclusion, the present study illustrated that exogenous application of Si effectively mitigated the adverse effects of Cd in raspberry plants by enhancing the growth, photosynthesis and antioxidative enzyme activities meanwhile decreasing the contents of MDA and recovery from Cd-induced ultrastructural alteration. Furthermore, Si-treatment enhances tolerance of raspberry plants to Cd toxicity through hampering Cd uptake and transportation and through reducing the soil available Cd, especially in the level of 200 mg kg⁻¹.

Declarations

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Figures

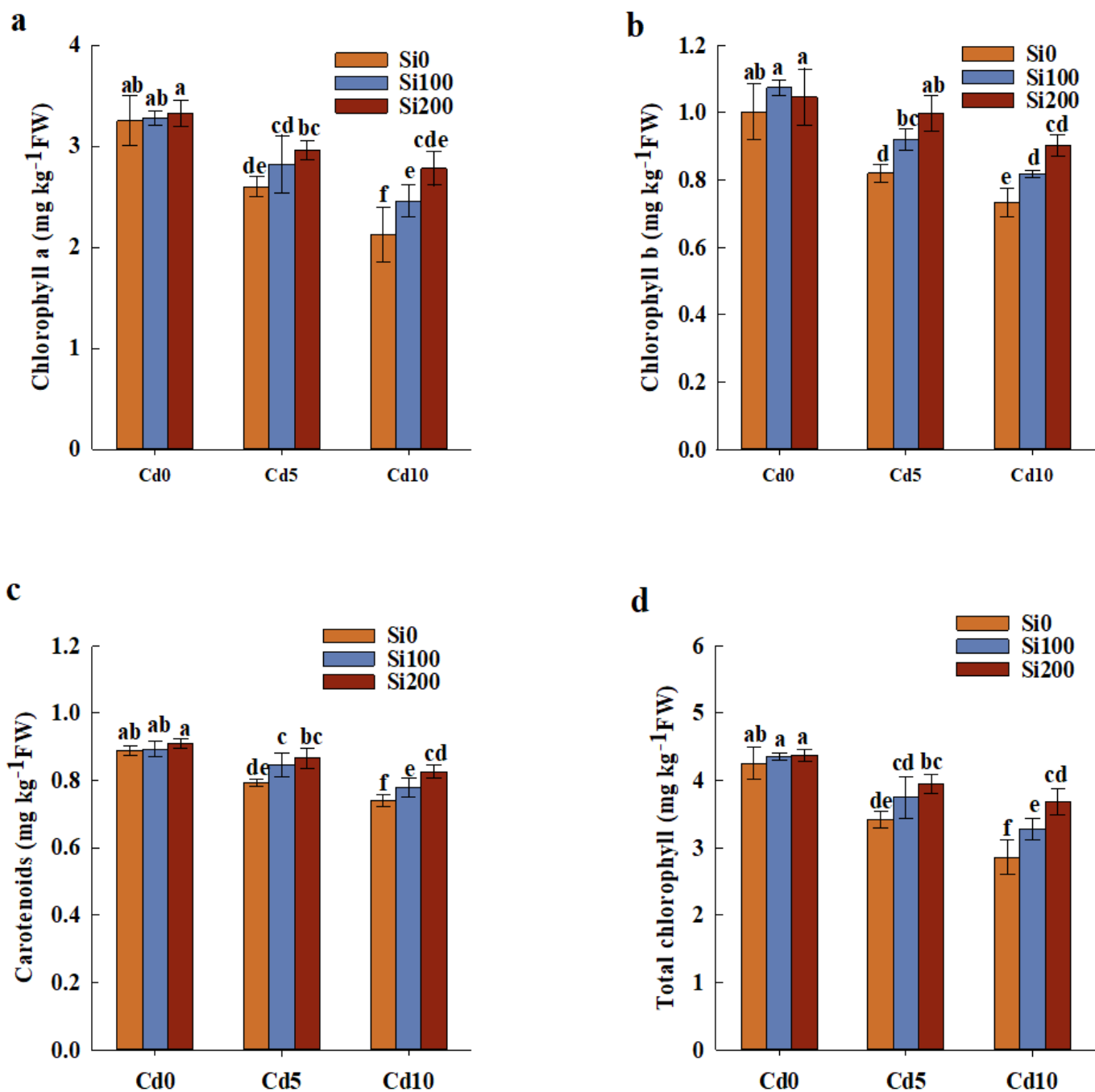


Figure 1

Effects of Si on the content of chlorophyll a (a), chlorophyll b(b), carotenoids (c) and total chlorophyll (d) in raspberry plants under Cd stress.Means±SD (n=3) with different letters in the same column indicate significant difference by Duncan's multiple range test (P <0.05) at different treatments.

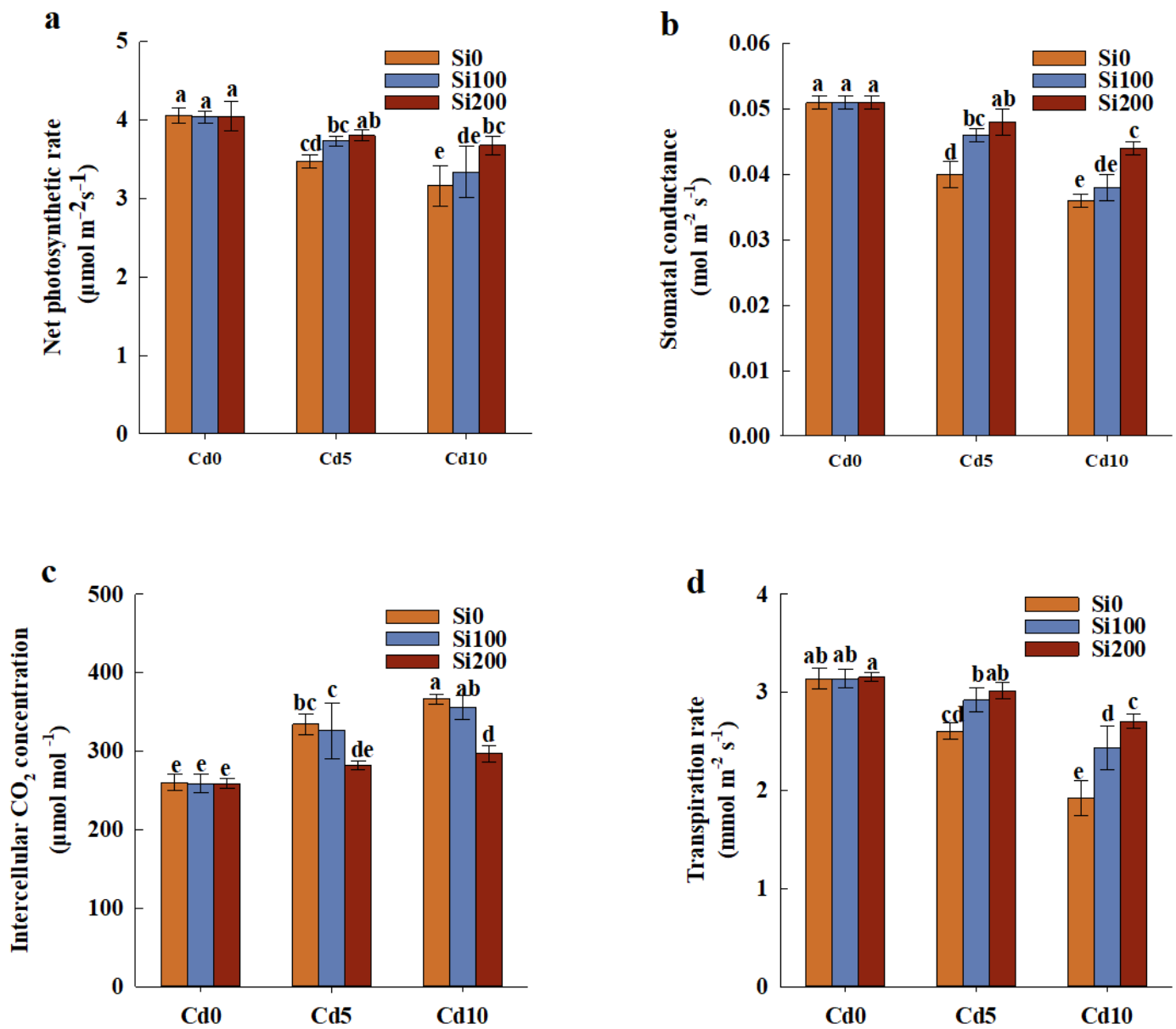


Figure 2

Effects of Si on net photosynthesis rate (P_n ; a), stomatal conductance (G_s ; b), intercellular CO_2 concentration (C_i ; c) and transpiration rate (T_r ; d) in raspberry plants under Cd stress. Means $\pm$ SD (n=3) with different letters in the same column indicate significant difference by Duncan's multiple range test ($P < 0.05$) at different treatments.

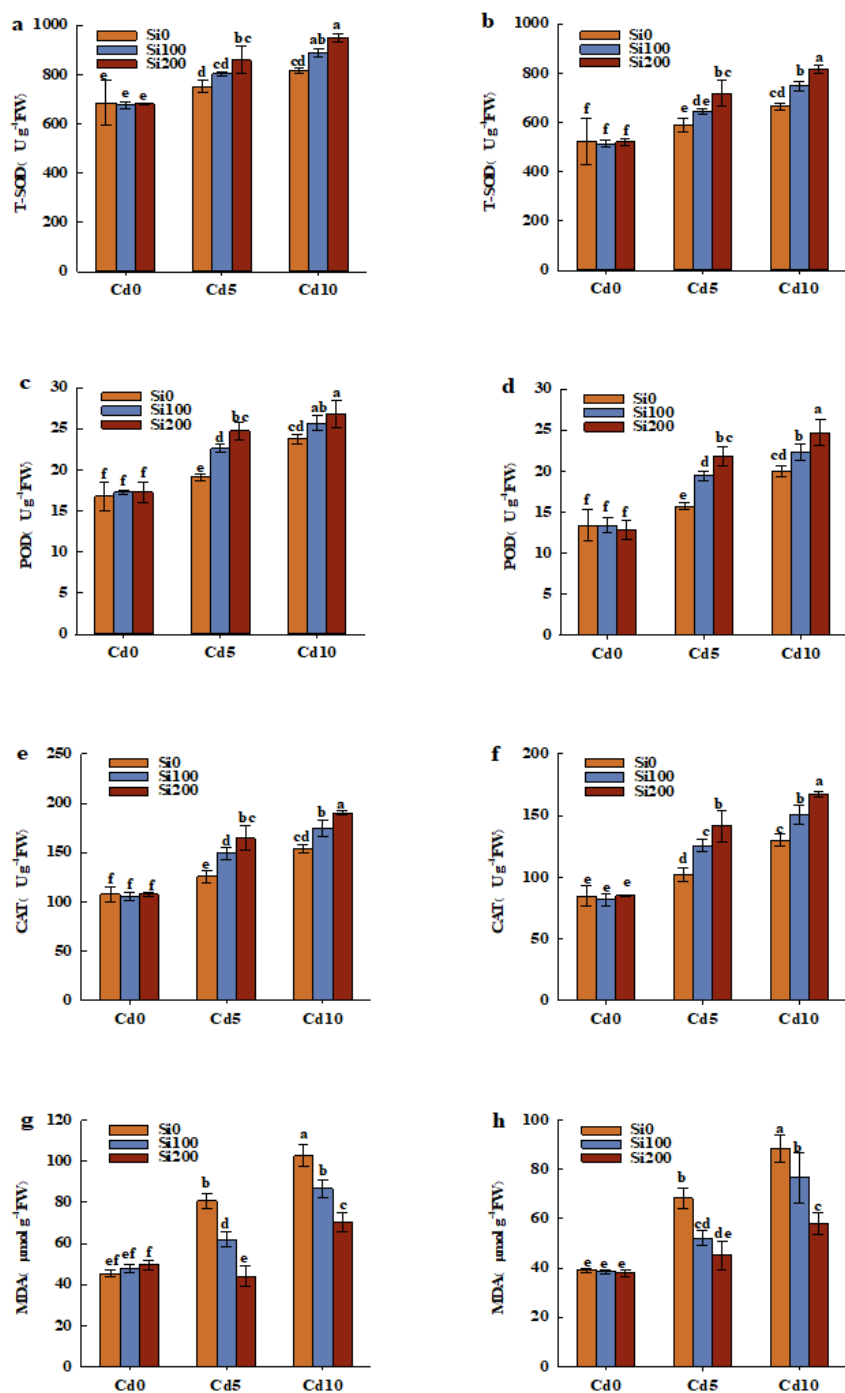


Figure 3

Effects of Si on activities of antioxidant enzymes superoxide dismutase (SOD; a: leaf; b: root), peroxidase (POD; c: leaf; d: root), catalase (CAT; e: leaf; f: root) activities and malondialdehyde MDA; g: leaf; h: root) in raspberry plants under Cd stress. Means±SD (n=3) with different letters in the same column indicate significant difference by Duncan's multiple range test (P < 0.05) at different treatments.

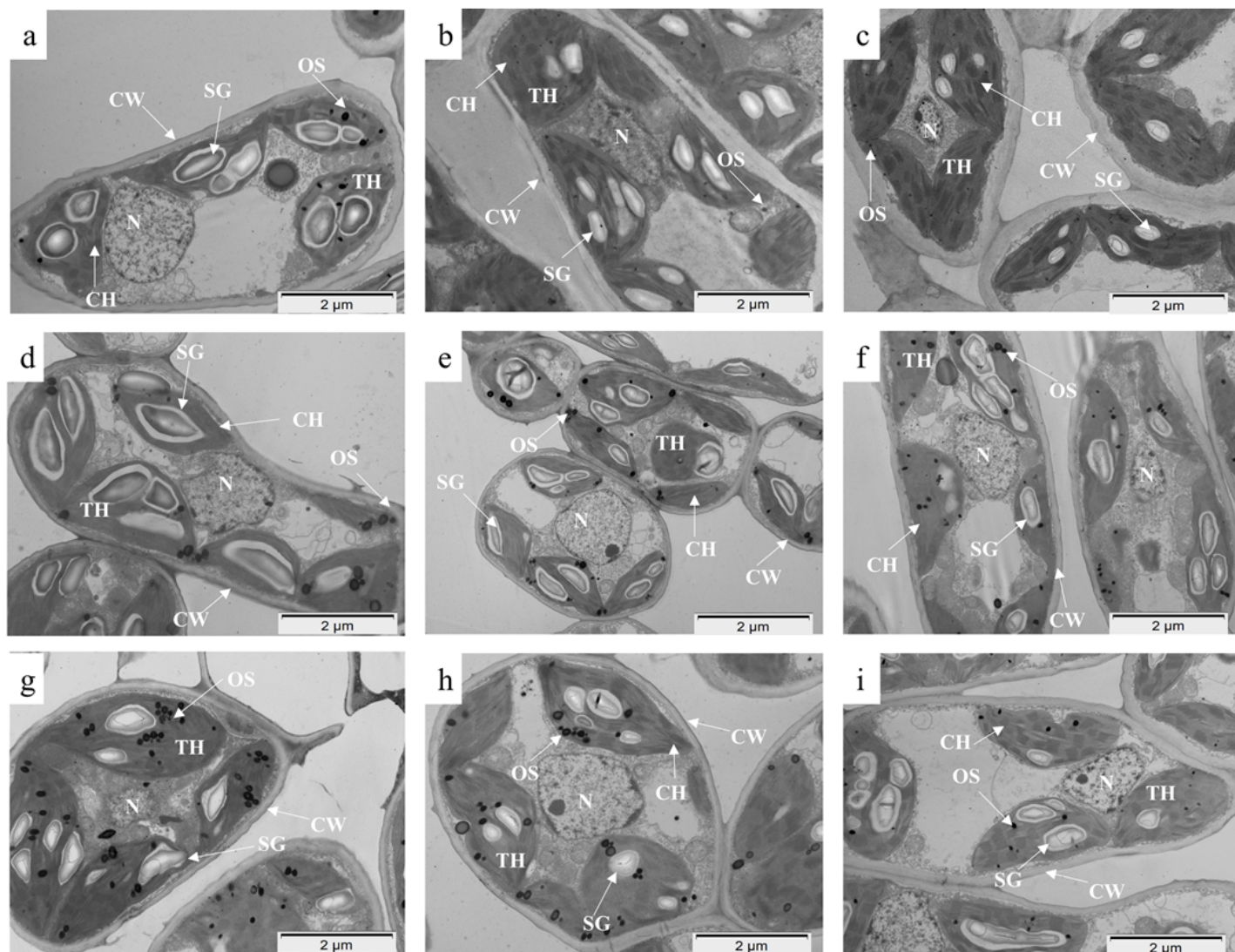


Figure 4

Transmission electron micrograph of mesophyll cells in raspberry plants under Cd stress. Control(a), Si100(b), Si200(c), Cd5(d), Cd5+Si100(e), Cd5+Si200(f), Cd10(g), Cd10+Si100(h), Cd10+Si200(i). Abbreviations: CW, cell wall; N, nucleus; CH, chloroplast; SG, starch granules; OS, osmiophilic globule; TH, thylakoid membranes. Scale bar = 2μm.

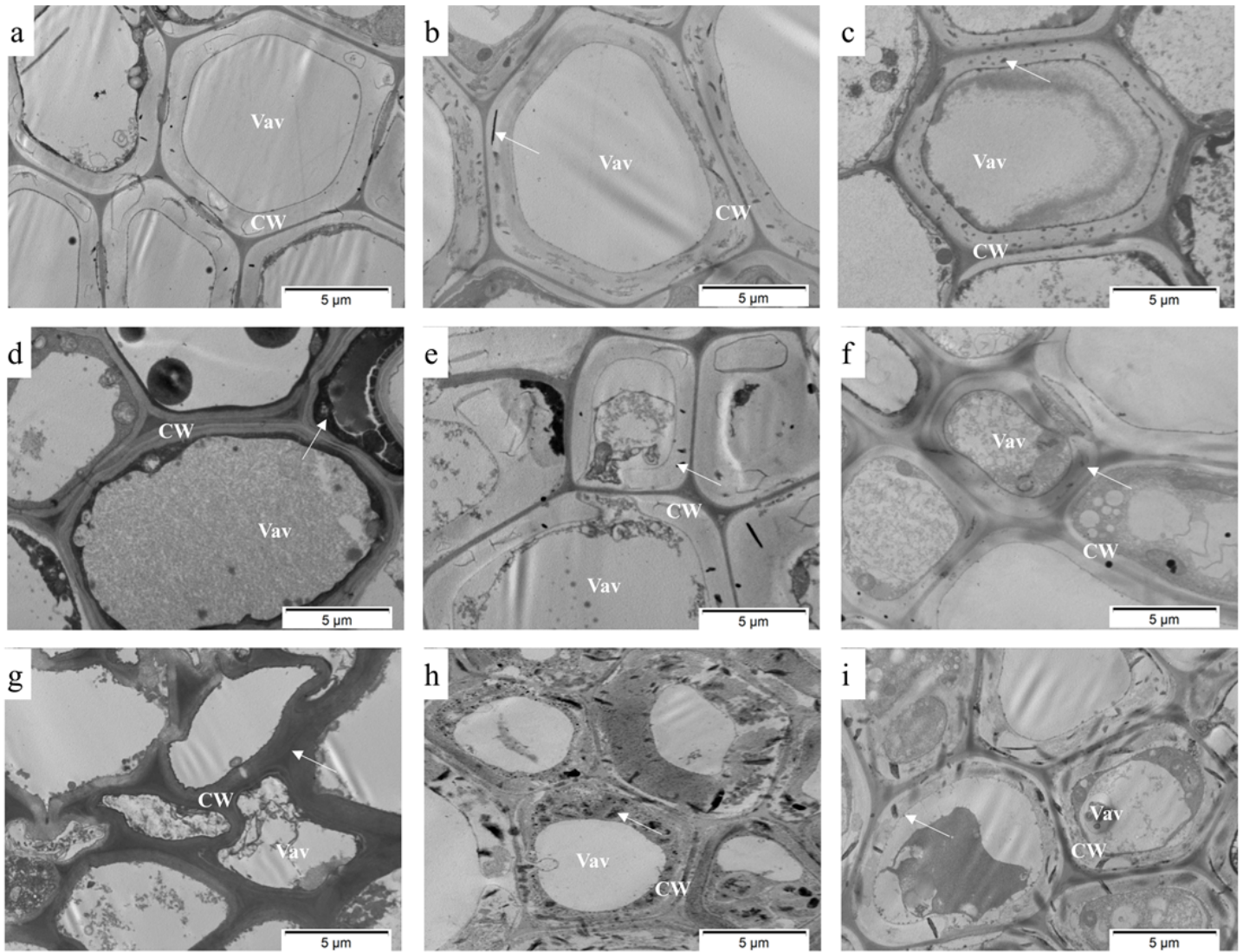


Figure 5

Transmission electron micrograph of root tip cells in raspberry plants under Cd stress. Control(a), Si100(b), Si200(c) Cd5(d), Cd5+Si100(e), Cd5+Si200(f), Cd10(g), Cd10+Si100(h), Cd10+Si200 (i). Abbreviations: CW, cell wall; Vac, vacuole; white arrow, electron dense material. Scale bar = 5μm.