1 Selenium Supply Alters Subcellular Distribution and Chemical Forms of Cadmium and Expression of Transporter Genes Involved in Cadmium Uptake and Translocation in Winter Wheat (Triticum Aestivum)

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Abstract

Background

Cadmium (Cd) accumulation in crops will affect the yield and quality of crops, and also harm human health. The application of selenium (Se) can reduce the absorption and transport of Cd in winter wheat.

Results

The result showed that increasing Se supply significantly decreased Cd concentration and accumulation in shoots and roots of winter wheat, and the root to shoot translocation of Cd. The Se supply increased the root length, surface area and root volume, but decreased the root average diameter. Increasing Se supply significantly decreased Cd concentration in cell wall, soluble fraction and cell organelle in roots and shoots. An increase of Se supply inhibited Cd distribution in the organelle of shoot and root, but enhanced Cd distribution in the soluble fraction of shoot and the cell wall of root. The Se supply also decreased the proportion of active Cd (ethanol-extractable (FE) Cd and deionized water-extractable (FW) Cd) in roots. In addition, the expression of \( TaN\text{ramp5-a, TaN}\text{ramp5-b, TaHMA3-a, TaHMA3-b} \) and \( TaHMA2 \) were significantly increased with the increase of Cd concentration in roots, and the expression of \( TaN\text{ramp5-a, TaN}\text{ramp5-b and TaHMA2} \) in roots were down-regulated by increasing Se supply, regardless of Se supply or Cd stress, respectively. The expression of \( TaHMA3-b \) in root was significantly down-regulated by \( Se_{10} \) treatment at both \( Cd_5 \) and \( Cd_{25} \) but up-regulated by \( Se_{5} \) treatment at \( Cd_{25} \). The expression of \( TaN\text{ramp5-a, TaN}\text{ramp5-b, TaHMA3-a, TaHMA3-b and TaHMA2} \) in shoot were down-regulated by increasing Se supply at \( Cd_{5} \), and \( Se_{5} \) treatment up-regulated the expression of those genes in shoot at \( Cd_{25} \).

Conclusions

The results confirm that Se application limit Cd accumulation in wheat via regulating subcellular distribution and the chemical forms of Cd in tissues of winter wheat, as well as the expression of \( TaN\text{ramp5-a, TaN}\text{ramp5-b and TaHMA2} \) in root.

Background

Cadmium (Cd) is one of the most dangerous heavy metals due to its detrimental effect on agricultural soil and potential harm to human health[1]. It is generally believed that plants are the major source of Cd uptake by human beings. Thus, Cd can harm human health through the enrichment effect of food chain. Wheat is not only one of the principal food in the north of China but also the most important grain crop in the world[2]. Cd-polluted wheat accumulation in humans may cause many diseases, such as anemia, osteoporosis, kidney damage and hypertension[3]. Therefore, it has become an urgent public health problem to reduce the accumulation of Cd in wheat and maintain food safety[4].
Although Cd has no essential biological function in plants, the accumulation of Cd in plants will produce obvious toxic effects, including destroying chlorophyll, inhibiting photosynthesis and crop growth and development, reducing yield and quality[5]. The intracellular and extracellular mechanisms for detoxification in plants have gradually developed in the process of adapting to heavy metal stress. Binding in the cell wall and the transfer to vacuoles may be associated with metal tolerance[6]. The toxicity and migration ability of heavy metals are closely related to their chemical forms. This suggests that Cd chemical forms could affect the movement of Cd in plants and be one of the major mechanisms of heavy metal detoxification[7]. The total amount of Cd entering plants is determined by the absorption capacity of Cd in root. Cd in soil is absorbed by plant root and transported to other parts through transporters for some essential elements, such as manganese (Mn), zinc (Zn) and iron (Fe) [8]. At least seven families of transporters participate in the Cd transport in plants, including natural resistance-associated macrophage proteins (NRAMP), heavy metal ATPases (HMA), ATP-binding cassette transporters(ABC), Zrt/Irt-like proteins (ZIP), H⁺/cation exchanger (CAX), LCT transporter and cation efflux family (CE) [9]. Se is an essential trace element for humans, animals and plants[10]. The Se can promote the growth and development of plants by improving antioxidant function and regulating photosynthesis. In addition, Se plays a vital role in plant resistance to adversity stress and the alleviation of the toxicity of heavy metals[11]. The Se is also a beneficial element for human, which can maintain human health by improving immunity, resisting aging and reducing cancer risk[12]. In recent years, many research results showed that Se and Cd in plants are antagonistic. Sun et al. [13] found that Se could reduce Cd concentration in maize and promote maize growth under Cd stress. Wan et al. [14] also reported that the translocation of Cd from root to shoot reduced effectively with the increase of Se supply in rice seedlings. In addition, Ahmad et al. [15] found that Se reduces Cd toxicity by regulating antioxidative system in Brassica juncea. Shanker et al.[16] revealed that Se and Cd can be combined to form a complex, thus reducing the toxicity of Cd. These studies suggest that applying Se fertilizer is an effective measure to reduce Cd accumulation in plants.

The aims of the present study was to: i) re-examine the effects of different Se supply rates on Cd uptake and translocation; ii) investigate the subcellular distribution and chemical forms of Cd in response to different Se supply rates; and iii) investigate the expression of Cd transporter genes regulated by different Se supply rates, under two levels of Cd stress using a hydroponic trial. Our results will contribute to a better understanding of the mechanism of Se inhibiting Cd uptake and translocation in winter wheat.

**Methods**

**Plant material and experimental designs**

Winter wheat (Triticum aestivum cv Zhengmai379, obtained from Henan Agricultural High Tech Group Co., Ltd.) seeds were sterilized for 15 min with 10% NaClO, rinsed by deionized water, and then cultured at 25 °C for 5 days. Then, 20 seedlings of the same size were transferred to the plastic pot containing 4L nutrient solution. The composition of nutrient solution was: 6.0 mM KNO₃, 4.0 mM Ca(NO₃)₂·4H₂O, 2.0 mM MgSO₄·7H₂O, 1.0 mM NaH₂PO₄·2H₂O, 100μM EDTAFe, 46μM H₃BO₃, 9 μM MnCl₂·4H₂O, 0.8 μM
ZnSO$_4$$\cdot$7H$_2$O, 0.3 μM CuSO$_4$$\cdot$5H$_2$O, and 0.09 μM Na$_2$MoO$_4$$\cdot$2H$_2$O. The Cd was added to the solution as CdCl$_2$ at two levels: 5 and 25 μM and Se was added as Na$_2$SeO$_3$ at three levels: 0, 5, and 10 μM after seedlings transferred for one week. Six treatments were included: Cd$_5$Se$_0$, Cd$_5$Se$_5$, Cd$_5$Se$_{10}$, Cd$_{25}$Se$_0$, Cd$_{25}$Se$_5$ and Cd$_{25}$Se$_{10}$. Each treatment was replicated three times. The quarter and half strength nutrient solutions were provided at the first and second weeks, respectively, followed by full-strength nutrient solutions. The greenhouse conditions were as follows: relative humidity 70%, 14h light / 10h dark at 25 / 18 °C, light intensity of 400 μmol m$^{-2}$s$^{-1}$.

The seedlings were harvested after 21 days, and the shoot and root were separated. Ten seedlings were dried in an electric oven at 60°C to analyze the Cd concentration in plant issues. The others were frozen in liquid nitrogen immediately, and then stored at -80°C for further subcellular fractions, chemical forms and gene expression analysis.

**Determination of Cd concentration**

The Cd concentration in plant issues were determined by the method of Liu et al. [17]. Dry samples were powdered and digested in the mixture of HNO$_3$:HClO$_4$ (4:1, v/v). The Cd concentration in solution were determined using the same atomic absorption spectrophotometer (ZEEnit 700, Analytik Jena AG, Germany).

**Determination of root morphology**

After 14 days of seedling growth, one seedling from each pot was taken for the root morphological analysis. The root length, root surface area, root volume, and average root diameter of wheat were measured by using the root imaging analysis software WinRHI-ZO Version 2009 PRO (Regent Instruments, Quebec City, Canada).

**Determination of Subcellular fractions**

According to Zhao et al. [18], frozen samples were homogenized in pre-cold extraction buffer containing 50 mM Tris-HCl (pH 7.5), 1.0 mM dithiothreitol (C$_4$H$_{10}$O$_2$S$_2$) and 250 mM sucrose at the ratio of 1:20 (w/v). The mixtures were centrifuged at 924×g for 15min and the cell wall fraction was obtained in the residue. After centrifuging the supernatant at 20,000×g for 45 min, the supernatant solution and precipitate were called soluble fraction and cell organelle fraction, respectively. All steps were carried out at 4 °C. The mixture of HNO$_3$:HClO$_4$ (4:1, v/v) was used for wet digestion of different fractions and Cd concentration in digestion solution was determined using the flame atomic absorption spectrophotometer (ZEEnit 700, Analytik Jena AG, Germany).

**Extraction of Cd in different chemical forms**

According to Zhang et al.[19], six kinds of Cd with different chemical forms were extracted. The extractive sequence was as follows: (1) 80% ethanol (FE-Cd), extracting inorganic Cd and aminophenol Cd; (2)
deionized water (FW-Cd), extracting water-soluble Cd of organic acid complexes and Cd(H₂PO₄)₂; (3) 1 M NaCl (FNaCl-Cd), extracting Cd integrated with pectate and protein; (4) 2% acetic acid (FHAC-Cd), extracting insoluble CdHPO₄, Cd₃(PO₄)₂ and other Cd-phosphate complexes; (5) 0.6 M HCl (FHCl-Cd), extracting oxalate acid-bound Cd; (6) Cd in residues (FC-Cd).

About 0.5 g of frozen samples were added to the extraction solution at the ratio of 1:10 (w/v), and then it was shaken at 25°C for 22 h, and centrifuged at 5000×g for 10 min. The precipitate was re-suspended with the same extractive solution twice, shaken at 25 °C for 2 h, and then centrifuged at 5000×g for 10 min. Pooled the supernatant after three centrifugations, and evaporated to 1-2 mL on an electric plate. Each form of Cd was digested with HNO₃:HClO₄ (4:1, v/v) and Cd concentration were analysed by flame atomic absorption spectrophotometer (ZEEnit 700, Analytik Jena AG, Germany).

Expression of TaNramp5-a, TaNramp5-b, TaHMA3-a, TaHMA3-b, TaHMA2

Total RNA was extracted from seedling shoot and root, and then used for first-strand cDNA synthesis using a PrimeScript™ RT reagent Kit (TakaRa) in accordance with the manufacturer's protocol. The expression was determined with TB green premix Ex Taq™ II (TakaRa). Relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. The primers for TaHMA2 was obtained from Tan et al.[20], and the primers for TaNramp5-a, TaNramp5-b, TaHMA3-a and TaHMA3-b were designed by GenScript Real-time PCR (TaqMan) Primer Design Online (https://www.genscript.com.cn/ based on the mRNA sequences obtained from the Ensembl database (http://plants.ensembl.org/). Primers for the genes of interest and reference genes are detailed in Table S1.

Statistics analysis

The main effects and interactions of Cd and Se were statistically examined by two-way ANOVA using SPSS 7.05 software (Chicago, USA). Tukey-test was used for multiple comparisons at a 5% significance level ($P<0.05$).

Results

Cd concentrations, accumulation and migration rate

The Cd and Se treatments had significant effects on Cd concentration and accumulation in shoot and root as well as Cd migration rate from root to shoot ($P<0.01$; Table S2); Their interaction had significant effects on the Cd concentration ($P<0.01$; Table S2) and migration rate from root to shoot ($P<0.05$; Table S2).

The Cd concentration in root was higher than that in shoot (Fig. 1A and B). At Se₀ treatment, Cd concentration in shoot and root was significantly increased by increasing Cd stress level; at Se₅ and Se₁₀ treatments, Cd concentration in root was also significantly increased by increasing Cd stress level. Compared with Se₀, Se₅ and Se₁₀ significantly decreased the shoot Cd concentration at each Cd stress level.
level, with the range of decreased degree from 27.6% to 67.7% (Fig. 1A). Similarly, Se$_{5}$ and Se$_{10}$ significantly decreased the root Cd concentration at each Cd stress level, with the range of decreased degree from 18.6% to 53.6%, except for the no obvious effect of Se$_{5}$ on the root Cd concentration at Cd$_{5}$ (Fig. 1B).

The Cd accumulation in root was also higher than that in shoot (Fig. 1C and D). At Se$_{5}$ and Se$_{5}$ treatments, with the increase of Cd stress level, Cd accumulation in shoot was significantly decreased. Compared with Se$_{0}$, Se$_{5}$ and Se$_{10}$ significantly decreased the shoot Cd accumulation at each Cd stress level, with the range of decreased degree from 33.3–71.6% (Fig. 1C). The Se$_{10}$ significantly decreased the root Cd accumulation at each Cd stress level by 46.9% and 61.5% (Fig. 1D).

Compared with Cd$_{5}$ treatment, Cd migration rate from root to shoot was significantly decreased by Cd$_{25}$ treatment at Se$_{5}$ (Fig. 2). At each Cd stress level, Se$_{5}$ and Se$_{10}$ significantly reduced the Cd migration rate from root to shoot, with the range of decreased degree from 18.8% to 30.3%.

**Root morphology**

The Cd, Se treatments and their interaction had significant effects on root length, root total surface area and root volume ($P$< 0.01; Table S3). The Se treatments had significant effects on the average root diameter ($P$< 0.01; Table S3).

The root length, root volume and root surface area were reduced significantly with increasing Cd stress (Fig. 3A, C and D). At Cd$_{5}$, Se$_{5}$ and Se$_{10}$ significantly increased the root length, surface area and root volume, but decreased the average root diameter in winter wheat, with the range of decreased or increased degree from 12.3% to 89.2%. At Cd$_{25}$, Se$_{5}$ and Se$_{10}$ significantly reduced the average root diameter by 11.0% and 19.3%, respectively; but Se$_{5}$ and Se$_{10}$ significantly increased the root volume by 57.2% and 46.9%, respectively.

**Cd subcellular fraction and distribution**

The Cd, Se treatments and their interaction had significant effects on subcellular fractions of Cd in tissues of wheat seedlings ($P$< 0.01; Table S4).

The Cd concentration in each fraction of shoot and root was significantly increased by increasing Cd stress level, except for the Cd concentration in cell wall of shoot at Se$_{10}$, that in soluble fraction and cell organelle of shoot at Se$_{5}$ and Se$_{10}$, and that in cell organelle of root at Se$_{10}$ (Table 1). At Cd$_{5}$, Cd concentration in cell wall, soluble fraction and cell organelle of shoot were significantly decreased by Se$_{5}$ and Se$_{10}$, with the range of decreased degree from 19.0–43.2%; Se$_{10}$ significantly decreased the Cd concentration in soluble fraction and cell organelle of root by 31.3% and 49.3%, respectively. At Cd$_{25}$, Se$_{5}$ and Se$_{10}$ significantly decreased the Cd concentration in cell wall, soluble fraction and cell organelle of shoot and root, with the range of decreased degree from 17.9–65.9%.
Table 1
Subcellular fractions of Cd in tissues of winter wheat (*Triticum aestivum* cv Zhengmai379) seedlings grown with low (0 µM), medium (5 µM), or high (10 µM) Se supply under low (5 µM) or high (25 µM) Cd stress for 21 d.

| Treatment | Shoot/(mg·kg⁻¹ DW) | Root/(mg·kg⁻¹ DW) |
|-----------|---------------------|-------------------|
|           | Cell wall | Soluble fraction | Cell organell e | Cell wall | Soluble fraction | Cell organell e |
| Cd (µM·L⁻¹) | Se (µM·L⁻¹) |            |                |          |                |                |
| 5         | 0          | 3.53 ± 0.07b   | 11.0 ± 1.05b   | 2.16 ± 0.32b | 21.3 ± 0.71d | 138 ± 1.65d   | 10.7 ± 0.82bc |
|           | 5          | 2.47 ± 0.14c   | 8.92 ± 0.38 cd | 1.60 ± 0.04 cd | 19.9 ± 1.83d | 128 ± 4.40d   | 8.57 ± 0.80 cd |
|           | 10         | 2.10 ± 0.12c   | 7.06 ± 0.33e  | 1.14 ± 0.23d  | 16.8 ± 1.45d | 95.1 ± 3.95e  | 5.39 ± 0.53e  |
| 25        | 0          | 4.61 ± 0.051a  | 14.2 ± 0.84a   | 3.50 ± 0.07a  | 68.8 ± 0.64a | 352 ± 21.9a   | 22.2 ± 1.55a  |
|           | 5          | 3.78 ± 0.04b   | 10.0 ± 0.46bc  | 1.93 ± 0.11bc | 53.1 ± 3.57b | 285 ± 8.39b   | 13.3 ± 1.58b  |
|           | 10         | 2.36 ± 0.18c   | 7.45 ± 0.25de  | 1.41 ± 0.16d  | 45.7 ± 0.66c | 188 ± 1.86c   | 7.58 ± 0.67de |

Values are means of three independent replicates (± sd). For each trait, means followed by different letters are significantly different from each other according to two-way ANOVA followed by Turkey multiple comparison (P< 0.05).

In both of shoot and root, the proportion of Cd in soluble fraction was higher than that in cell organelle or cell wall (Fig. 4). Cd proportion in cell organelle of shoot at Se₀ and Se₁₀, and that in cell wall of shoot at Se₅ and Se₁₀, and that in cell wall of root at each Se level was increased by increasing Cd stress level; Cd proportion in soluble fraction of shoot and root at each Se level, and that in cell wall of shoot at Se₀, and that in cell organelle of root at each Se level was decreased by increasing Cd stress level. Se supply decreased Cd proportion in cell organelle of shoot and root at two Cd levels, with the range of decreased degree from 4.65–38.0% (Fig. 4A and B). But Se supply increased Cd proportion in soluble fraction of shoot and Cd proportion in cell wall of root, with the range of increased degree from 1.60–21.9%. Se supply decreased Cd proportion in cell wall of shoot at Cd₅ but increased its proportion at Cd₂₅. Se₅ increased but Se₁₀ decreased Cd proportion in soluble fraction of root.

**Cd chemical forms and distribution**

The Cd treatments had significant effects on FE-Cd, FNaCl-Cd, FHAC-Cd and FC-Cd concentration in shoot as well as the concentration of FE-Cd, FW-Cd, FNaCl-Cd, FHAC-Cd, FHCl-Cd and FC-Cd in root (P< 0.01 or P< 0.05; Table S5). Se treatments had significant effects on FE-Cd, FW-Cd, FNaCl-Cd and FHAC-Cd concentration in shoot as well as the concentration of FE-Cd, FW-Cd and FNaCl-Cd in root (P< 0.01 or P<
There was a significant interactive effect of Se and Cd on FE-Cd concentration in shoot as well as the concentration of FE-Cd, FW-Cd, FNaCl-Cd and FHAC-Cd in root ($P < 0.01$; Table S5).

At Se$_0$, FE-Cd and FHAC-Cd concentration in shoot as well as the concentration of FE-Cd, FW-Cd, FNaCl-Cd, FHAC-Cd and FHCl-Cd in root was significantly increased by increasing Cd stress level (Table 2); At Se$_5$, with the increase of Cd stress level, FE-Cd concentration in shoot as well as the concentration of FE-Cd, FW-Cd, FNaCl-Cd and FHAC-Cd in root was markedly increased; At Se$_{10}$, with the increase of Cd stress level, FE-Cd and FNaCl-Cd concentration in root was significantly increased; but at Se$_5$ and Se$_{10}$, FNaCl-Cd concentration in shoot was dramatically reduced by increasing Cd stress level. At Cd$_5$, Se$_5$ and Se$_{10}$ significantly increased FE-Cd concentration in root but decreased FNaCl-Cd in shoot and FW-Cd in root, with the range of decreased or increased degree from 25.2% to 60.6% (Table 2). At Cd$_{25}$, Se$_5$ significantly increased FE-Cd concentration in shoot but decreased FHAC-Cd in shoot and FW-Cd, FNaCl-Cd in root; Se$_{10}$ significantly decreased FE-Cd, FW-Cd, FNaCl-Cd and FHAC-Cd in shoot and root, with the range of decreased or increased degree from 10.1% to 82.0%.
Table 2
Chemical forms of Cd in tissues of winter wheat (Triticum aestivum cv Zhengmai379) seedlings grown with low (0 µM), medium (5 µM), or high (10 µM) Se supply under low (5 µM) or high (25 µM) Cd stress for 21 d.

| Tissues | Treatment | Cd/mg·kg⁻¹ DW |
|---------|-----------|---------------|
|         | Cd        | Se | FE  | FW  | FNaCl | FHAC | FHCl | FC  |
| Shoot   | 5         | 0  | 0.62 ± 0.02c | 1.27 ± 0.04ab | 19.78 ± 1.75a | 1.36 ± 0.24b | 0.10 ± 0.01a | 0.06 ± 0.01a |
|         | 5         | 0  | 0.91 ± 0.12bc | 1.35 ± 0.10ab | 14.79 ± 1.23b | 0.74 ± 0.12b | 0.11 ± 0.02a | 0.06 ± 0.01a |
|         | 10        | 0  | 0.80 ± 0.09bc | 1.14 ± 0.15ab | 12.41 ± 0.94b | 0.55 ± 0.08b | 0.10 ± 0.02a | 0.04 ± 0.01a |
|         | 25        | 0  | 1.28 ± 0.48b | 1.73 ± 0.53a | 14.51 ± 0.38b | 1.75 ± 0.16a | 0.13 ± 0.04a | 0.07 ± 0.02a |
|         | 5         | 0  | 2.33 ± 0.12a | 1.10 ± 0.32ab | 12.21 ± 0.44bc | 0.83 ± 0.22b | 0.10 ± 0.02a | 0.07 ± 0.02a |
|         | 10        | 0  | 0.51 ± 0.15c | 0.97 ± 0.19b | 9.59 ± 0.69c | 0.71 ± 0.09b | 0.13 ± 0.02a | 0.07 ± 0.02a |
| Root    | 5         | 0  | 13.69 ± 0.80e | 118.55 ± 3.97c | 58.36 ± 4.81de | 7.88 ± 1.73b | 0.65 ± 0.05b | 0.12 ± 0.03ab |
|         | 5         | 0  | 17.17 ± 1.28d | 80.45 ± 3.96d | 67.25 ± 3.35 cd | 12.46 ± 0.06b | 0.67 ± 0.08b | 0.14 ± 0.06ab |
|         | 10        | 0  | 21.91 ± 1.86c | 46.68 ± 3.13e | 56.12 ± 3.52e | 13.00 ± 0.91b | 0.87 ± 0.13b | 0.09 ± 0.04b |
|         | 25        | 0  | 66.29 ± 0.53a | 201.03 ± 5.11a | 92.93 ± 1.75a | 24.81 ± 4.66a | 3.09 ± 1.17a | 0.21 ± 0.05a |
|         | 5         | 0  | 63.23 ± 1.03a | 136.03 ± 3.56b | 83.57 ± 1.67b | 21.32 ± 4.03a | 1.57 ± 0.46ab | 0.12 ± 0.04ab |
|         | 10        | 0  | 40.81 ± 1.11b | 53.14 ± 3.53e | 71.91 ± 3.78c | 13.36 ± 1.69b | 1.90 ± 0.73ab | 0.16 ± 0.02ab |

Values are means of three independent replicates (± sd). For each trait, means followed by different letters are significantly different from each other according to two-way ANOVA followed by Turkey multiple comparison (P< 0.05).

The Cd proportion in each chemical forms of shoot and root was significantly increased by increasing Cd stress level, except for FNaCl-Cd of shoot and root, FW-Cd of root, FHAC-Cd of root at Se₁₀, FE-Cd of shoot.
at Se$_{10}$ and FW-Cd of shoot at Se$_5$ (Fig. 5). In root, Se$_5$ and Se$_{10}$ increased the proportion of FE-Cd, FNaCl-Cd and FHAC-Cd, with the range of increased degree from 9.38% to 135%; but Se$_5$ and Se$_{10}$ decreased the proportion of FW-Cd, with the range of decreased degree from 14.1% to 43.4%. In shoot, Se supply increased the proportion of FW-Cd and FE-Cd at Cd$_5$, but decreased FW-Cd and FE-Cd proportion at Cd$_{25}$, FNaCl-Cd and FHAC-Cd proportion at two Cd level, except for FE-Cd at the treatment of Cd$_{25}$Se$_5$, and FNaCl-Cd at the treatment of Cd$_{25}$Se$_{10}$, with the range of increased or decreased degree from 1.48% to 107%.

**Expression of TaNramp5-a, TaNramp5-b, TaHMA3-a, TaHMA3-b and TaHMA2**

The Cd, Se treatments and their interaction had significant effects on the transcript levels of TaNramp5-a, TaNramp5-b, TaHMA3-a, TaHMA3-b, TaHMA2 in shoot and root ($P < 0.05$ or $P < 0.01$; Table S6). The transcript levels of TaNramp5-a, TaNramp5-b, TaHMA3-a, TaHMA3-b and TaHMA2 in root were higher than those in shoot, except for the transcript level of TaHMA2 in the treatments of Cd$_{25}$Se$_5$ and Cd$_{25}$Se$_{10}$ (Fig. 6). In root, the transcript levels of TaNramp5-a, TaNramp5-b, TaHMA3-a and TaHMA3-b were significantly increased with the increase of Cd stress level; increasing Cd stress significantly increased the transcript level of TaHMA2 at Se$_0$ but decreased that at Se$_5$ and Se$_{10}$ (Fig. 6A, C, E, G and I). In shoot, increasing Cd stress significantly decreased the transcript levels of TaNramp5-b and TaHMA2 at Se$_0$, but increased the five genes transcript levels at Se$_5$ as well as the transcript levels of TaHMA3-a, TaHMA3-b and TaHMA2 at Se$_{10}$ (Fig. 6B, D, F, H and J). At Cd$_5$, Se$_{10}$ significantly decreased the transcript levels of TaNramp5-a, TaNramp5-b, TaHMA3-b and TaHMA2 in root as well as the transcript levels of TaHMA3-b and TaHMA2 in shoot, and Se$_5$ and Se$_{10}$ significantly decreased the transcript levels of TaNramp5-a, TaNramp5-b and TaHMA3-a in shoot. At Cd$_{25}$, the transcript levels of TaNramp5-a, TaNramp5-b and TaHMA2 in root were significantly decreased by both of Se$_5$ and Se$_{10}$ treatments; Se$_5$ significantly decreased the transcript level of TaHMA3-a in root, but increased TaHMA3-b transcript level in root and the five genes transcript levels in shoot; Se$_{10}$ significantly decreased the TaHMA3-b transcript level in root and TaNramp5-a transcript level in shoot, but increased transcript levels of TaHMA3-a and TaHMA2 in shoot.

**Discussion**

**Se inhibits Cd absorption via altering root morphology in winter wheat**

In our study, Se supply decreased Cd concentration and accumulation in both shoot and root (Fig. 1), indicating Se supply could inhibit Cd absorption in winter wheat. Huang et al. [21] found that Se application reduced Cd concentration in brown rice via a pot experiment, and Lin et al. [22] reported that Se decreasing the toxicity and accumulation of Cd in rice was related to the reduced Cd uptake. Plants absorb nutrients mainly through the root system [23]. Many studies showed that Cd stress could lead to the short root length, thick root diameter and reduced lateral root [24]. Our results observed that Se alleviated the toxic effect of Cd on the root growth of winter wheat, especially at low Cd stress, showing the increased root length, root surface area, root volume and the decreased root diameter by Se supply.
(Fig. 3). However, Ding et al. [25] found that the addition of 0.8 mg L$^{-1}$ Se to the treatments containing 4 mg L$^{-1}$ Cd increased the root length, surface area, volume, and average diameter of rice. The root morphology have a great influence on the absorption of minerals[5]. And the fine root are the most active part of the root system for mineral absorption[26]. Nazar et al. [27] also noted that plant nutrients such as iron (Fe), manganese (Mn) and zinc (Zn) and Cd compete for the same transporters. Therefore, the inhibited Cd uptake by Se application in this experiment may be related to the decreased root diameter and the increased mineral nutrient uptake by root.

**Se inhibits Cd transport via altering the distribution of Cd in subcellular fraction, chemical forms in tissues of winter wheat**

Our study suggested Se$_5$ and Se$_{10}$ significantly decreased Cd migration rate from root to shoot, and Cd concentration in cell wall, soluble fraction and cell organelle of shoot and root (Table 1 and Fig. 2). The decreased Cd concentration in subcellular fraction was due to the decreased Cd concentration in winter wheat by Se supply. It was also suggested that most of Cd accumulated in the soluble fraction, followed by that in the cell wall (Table 1 and Fig. 4). Our results are consistent with the results of Li et al. [28], who found that the majority of Cd was compartmentalized in the soluble fraction (53–75%) and bound to the cell wall (19–42%) in *Agrocybe aegerita*. Cd in soluble fraction and cell wall is easily chelated and fixed by organic substances, so it is difficult to transfer to other parts[29]. Li et al. [30] found that Cd in the soluble fraction of wheat root tended to combine with heat-stable protein (HSP), thus reducing the mobility and toxicity of Cd. In addition, vacuole (involved in the soluble fraction) is considered to accumulate the greatest amount of Cd and is the place where waste and by-products are accumulated [31]. Heavy metals can be separated in vacuoles through bounding with various proteins, organic acids and organic bases [32]. In our study, Se supply enhanced Cd accumulation in soluble fraction of shoot (Fig. 4A), indicating that Se supply could inhibit Cd migration to other organs thus to alleviate the Cd toxicity. Cell wall fraction can bind Cd ions reduce the transport to other parts, which is the first barrier to protect the protoplast from Cd toxicity[33]. Cd proportions in cell wall of root were increased by Se$_5$ and Se$_{10}$, respectively (Fig. 4B), suggesting Se supply enhanced Cd accumulation in root thus to inhibit Cd transport form root to shoot.

Different chemical forms of Cd have distinct migration capacity. For example, Compared with undissolved Cd phosphate (FHAC-Cd) and Cd oxalate (FHCl-Cd), inorganic and organic water-soluble Cd (FE-Cd and FW-Cd, respectively) have higher migration ability and greater harm to plant cells [7]. Some studies showed that FNaCl-Cd played an important role in the alleviation of Cd toxicity [18, 34]. In our study, Cd was mainly integrated with pectates and protein (FNaCl-Cd) in shoot and existed in the form of FW-Cd and FNaCl-Cd in root (Table 2 and Fig. 5). It indicates that Cd easily migrate from root to shoot in the water-soluble form but the toxicity of Cd also can be alleviated via converting Cd into undissolved pectate and protein-bound form. Qiu et al.[35] found that the majority of Cd in both the root and shoot of cabbage was in the extraction of 1 M NaCl. Some specific polar compounds contain hydroxyl or carboxyl which can combine with Cd to form a non-toxic complex[18]. Se supply significantly decreased the total
proportion of active Cd (FE-Cd and FW-Cd) but increased the proportion of FNaCl-Cd and FHAC-Cd in root, suggesting that Se supply reduced the mobility of Cd from root to shoot via promoting the transformation of Cd from active form to inactive form in root. The total proportion of active Cd (FE-Cd and FW-Cd) in shoot was decreased by high Se (Se$_{10}$) supply at Cd$_{25}$, suggesting that high level of Se supply could inhibit the mobility of Cd in shoot at high Cd stress level.

**Down-regulation of Cd transporter genes might be responsible for Se-decreased Cd accumulation in winter wheat**

It is widely believed that Cd enters plant root mainly through the Mn channel protein Nramp5 [36]. Nramp5 is a member of the Nramp family, located on the plasma membrane of plant roots [36]. In our study, the expression of TaNramp5-a and TaNramp5-b was found in both root and shoot, and that were significantly increased with the increase of Cd concentration (Fig. 6A, B, C and D), suggesting that Nramp5 might be involved in the absorption and transport of Cd in wheat plants. It is in agreement with the results of Ma et al.[37] showing that the expression of OsNramp5 was significantly increased with increasing Cd concentration. Tang et al.[38] and Sasaki et al.[36] observed that knockout of OsNramp5 can significantly reduce the Cd concentration in root and shoot of rice. In our study, Se supply significantly decreased the expression of TaNramp5-a and TaNramp5-b in shoot as Cd stress was low (Fig. 6B and D), indicating that Se supply might inhibit the remobilization of Cd in shoot. In addition, Se supply significantly decreased the expression of TaNramp5-a and TaNramp5-b in root (Fig. 6A and C), indicating that the down-regulation of TaNramp5-a and TaNramp5-b by Se supply might be helpful to decrease Cd uptake in wheat. Cui et al.[39] also found that Se pretreatment decreased the expression of OsNramp5 thus to inhibit Cd uptake.

Heavy metal ATPases (HMAs) is responsible for the transmembrane transport of cations and play an important role in Cd transport. HMA3 (heavy metal ATPase3), is located on the vacuole membrane in the root. And it is involved in the sequestration of Cd into the vacuoles of root cells, thereby decreasing the transport of Cd to the shoot and reducing the toxicity of Cd [40]. Sasaki et al.[41] reported that overexpression of OsHMA3 led to decreased root-to-shoot translocation of Cd. In our study, the expression of TaHMA3-a and TaHMA3-b was found in both root and shoot, and that were significantly increased with the increase of Cd concentration (Fig. 6E, F, G and H), suggesting that HMA3 might be responsible for the transport of Cd in wheat plants. Se supply down-regulated the expression of HMA3 in shoot at Cd$_5$ but up-regulated that at Cd$_{25}$ (Fig. 6F and H), also indicating that Se supply could inhibit the remobilization of Cd in shoot by enhancing the sequestration of Cd into the vacuoles as Cd stress was high. Cui et al. [39] also showed that Se pretreatment activated the expression of OsHMA3 thus to enhance the transport of Cd into vacuoles.

HMA2 (heavy metal ATPase2), which is homologous with HMA3 and belongs to the heavy metal ATPase family. HMA2 plays a role in the loading of Cd and Zn into xylem and get involved in the root-to-shoot translocation of Cd and Zn [20]. Our results showed that the expression of TaHMA2 was found in both root and shoot, and that were significantly increased with the increase of Cd concentration (Fig. 6I and J). It suggested that HMA2 might be involved in the transport of Cd in wheat plants. This is consistent with
the results of Tan et al. [20] showing that the overexpression of HMA2 in wheat and rice increased the root-shoot translocation of Zn/Cd. A recent report showed that La decreased Cd accumulation in wheat, which may be related to the TaHMA2 down-regulation [42]. In our study, Se supply significantly decreased the expression of TaHMA2 in root, indicating that the down-regulated TaHMA2 by Se supply might contribute to the inhibited Cd root-to-shoot translocation and final decreased Cd accumulation in shoot of winter wheat. The expression of TaHMA2 in shoot was significantly increased by Se supply at Cd_{25} (Fig. J), suggesting that Se supply might promote the remobilization of Cd in shoot by up-regulating the expression of TaHMA2 as Cd stress level was high.

**Conclusions**

Our results showed that TaNramp5, TaHMA3 and TaHMA2 might be responsible for the uptake and transport of Cd in wheat plants. Se supply could inhibit Cd absorption and root-to-shoot transport in winter wheat. Our results suggested that Se supply inhibit Cd absorption via reducing the root diameter and down-regulating the expression of TaNramp5. Meanwhile, Se supply inhibit the root to shoot translocation of Cd via promoting the distribution of Cd in cell wall and soluble fraction and in the inactive form in root, as well as down-regulating the expression of TaHMA2 in root of winter wheat.

**Abbreviations**

FE-Cd: Cd extracted by 80% ethanol; FW-Cd: Cd extracted by deionized water; FNaCl-Cd: Cd extracted by 1 M NaCl; FHAC-Cd: Cd extracted by 2% acetic acid; FHCl-Cd: Cd extracted by 0.6 M HCl; FC-Cd: Cd in residues.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.
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Authors' contributions

HL conceived and designed the experiments. PZ, SQ, CL, YW, XQ and HS performed the experiments. ZN and JZ analyzed the data. JZ wrote the paper. All authors have read and approved the submitted manuscript.

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Figures
Figure 1

Cd concentration and accumulation in shoot (A and C, respectively) and root (B and D, respectively) of winter wheat (Triticum aestivum cv Zhengmai379) seedlings.
Figure 2

Cd migration rate from the root to shoot of winter wheat seedlings (Triticum aestivum cv Zhengmai379) seedlings.

Figure 3

Root morphology parameters of winter wheat seedlings (Triticum aestivum cv Zhengmai379) seedlings.
Figure 4

Proportions of Cd in subcellular fractions of winter wheat seedlings (Triticum aestivum cv Zhengmai379) seedlings.

Figure 5

Proportions of Cd in chemical forms of winter wheat seedlings (Triticum aestivum cv Zhengmai379) seedlings.
**Figure 6**

The expression of TaNramp5-a, TaNramp5-b, TaHMA3-a, TaHMA3-b and TaHMA2 of winter wheat seedlings (*Triticum aestivum* cv Zhengmai379) seedlings.

**Supplementary Files**

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