**RESEARCH ARTICLE**                                                     **Open Access**

# [1]Selenium supply alters the subcellular distribution and chemical forms of cadmium and the 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 affects the yield and quality of crops and harms human health. The application of selenium (Se) can reduce the absorption and transport of Cd in winter wheat.

**Results:** The results showed that increasing Se supply significantly decreased Cd concentration and accumulation in the shoot and root of winter wheat and the root-to-shoot translocation of Cd. Se application increased the root length, surface area and root volume but decreased the average root diameter. Increasing Se supply significantly decreased Cd concentration in the cell wall, soluble fraction and cell organelles in root and shoot. An increase in Se supply inhibited Cd distribution in the organelles 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 root. In addition, the expression of *TaNramp5*-a, *TaNramp5*-b, *TaHMA3*-a, *TaHMA3*-b and *TaHMA2* significantly increased with increasing Cd concentration in root, and the expression of *TaNramp5*-a, *TaNramp5*-b and *TaHMA2* in root was downregulated by increasing Se supply, regardless of Se supply or Cd stress. The expression of *TaHMA3*-b in root was significantly downregulated by 10 µM Se at both the 5 µM and 25 µM Cd level but upregulated by 5 µM Se at the 25 µM Cd level. The expression of *TaNramp5*-a, *TaNramp5*-b, *TaHMA3*-a, *TaHMA3*-b and *TaHMA2* in shoot was downregulated by increasing Se supply at 5 µM Cd level, and 5 µM Se upregulated the expression of those genes in shoot at 25 µM Cd level.

**Conclusions:** The results confirm that Se application limits Cd accumulation in wheat by regulating the subcellular distribution and chemical forms of Cd in winter wheat tissues, as well as the expression of *TaNramp5*-a, *TaNramp5*-b and *TaHMA2* in root.

**Keywords:** Selenium, Cadmium, Subcellular distribution, Chemical forms, Gene expression, Wheat

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## Background

Cadmium (Cd) is one of the most dangerous heavy metals due to its detrimental effects on agricultural soil and potential harm to human health [1]. It is generally believed that plants are the major source of Cd uptake by humans. Thus, Cd can harm human health through the enrichment effect of the food chain. Wheat is not only one of the principal foods in northern 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 can produce obvious toxic effects, including destroying chlorophyll, inhibiting photosynthesis and crop growth and development, and 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 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 may affect the movement of Cd in plants and may 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 of the plant through transporters for essential elements, such as manganese (Mn), zinc (Zn) and iron (Fe) [8]. At least seven families of transporters participate in 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 the cation efflux family (CE) [9]. Se is an essential trace element for humans, animals and plants [10]. 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 adverse stress and the alleviation of the toxicity of heavy metals [11]. Se is also a beneficial element for humans and can maintain human health by improving immunity, resisting aging and reducing cancer risk [12]. In recent years, many research results have shown that Se and Cd in plants are antagonistic. Sun et al. [13] found that Se could reduce Cd concentrations in maize and promote maize growth under Cd stress. Wan et al. [14] also reported that the translocation of Cd from root to shoot decreased effectively with increasing Se supply in rice seedlings. In addition, Ahmad et al.

[15] found that Se reduces Cd toxicity by regulating the 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 way to reduce Cd accumulation in plants.

The aims of the present study were 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 mechanisms by which Se inhibits Cd uptake and translocation in winter wheat.

## Methods

### Plant materials and experimental design

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 with deionized water, and then cultured at 25 °C for 5 days. Then, 20 seedlings of the same size were transferred to plastic pots containing 4 L of nutrient solution. The composition of the nutrient solution was 6.0 mM $KNO_3$, 4.0 mM Ca $(NO_3)_2 \cdot 4H_2O$, 2.0 mM $MgSO_4 \cdot 7H_2O$, 1.0 mM $NaH_2PO_4 \cdot 2H_2O$, 100 μM EDTA-Fe, 46 μM $H_3BO_3$, 9 μM $MnCl_2 \cdot 4H_2O$, 0.8 μM $ZnSO_4 \cdot 7H_2O$, 0.3 μM $CuSO_4 \cdot 5H_2O$, and 0.09 μM $Na_2MoO_4 \cdot 2H_2O$. Cd was added to the solution as $CdCl_2$ at two levels: 5 and 25 μM, and Se was added as $Na_2SeO_3$ at three levels: 0, 5, and 10 μM after seedlings were transferred for 1 week. Six treatments were included: $Cd_5Se_0$, $Cd_5Se_5$, $Cd_5Se_{10}$, $Cd_{25}Se_0$, $Cd_{25}Se_5$ and $Cd_{25}Se_{10}$. Each treatment was replicated three times. The quarter- and half-strength nutrient solutions were provided in the first and second weeks, respectively, followed by full-strength nutrient solutions. The greenhouse conditions were as follows: relative humidity 70%, 14 h light/10 h dark at 25/18 °C, and 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 taken from each pot, and the root were soaked in 0.5 mM $CaCl_2$ and 2 mM MES solution for 30 min, washed with deionized water three times, and then dried in an electric oven at 60 °C until the weight was constant to analyze dry matter weight and Cd concentration in plant issues. The others were frozen in liquid nitrogen immediately and then stored at − 80 °C for further subcellular fraction, chemical form and gene expression analysis.

### Determination of Cd concentration

Cd concentrations in plant tissues were determined by the method described by Liu et al. [17]. Dry samples

were powdered and digested in a mixture of $HNO_3$: $HClO_4$ (4:1, v/v). The Cd concentration in solution was determined using a flame 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 root morphological analysis. The root length, root surface area, root volume, and average root diameter of the wheat samples were measured by using the root imaging analysis software WinRHI-ZO Version 2009 PRO (Regent Instruments, Quebec City, Canada).

### Determination of subcellular fractions
Using methods described by Zhao et al. [18], frozen samples were homogenized in precooled extraction buffer containing 50 mM Tris-HCl (pH 7.5), 1.0 mM dithiothreitol ($C_4H_{10}O_2S_2$) and 250 mM sucrose at a ratio of 1: 20 (w/v). The mixtures were centrifuged at $924 \times g$ for 15 min, and the cell wall fraction was obtained in the pellet. After centrifuging the supernatant at $20,000 \times g$ for 45 min, the supernatant solution and precipitate were called the soluble fraction and cell organelle fraction, respectively. All steps were carried out at $4\,^\circ C$. A mixture of $HNO_3$:$HClO_4$ (4:1, v/v) was used for wet digestion of the different fractions, and the Cd concentration in the digestion solution was determined using a flame atomic absorption spectrophotometer (ZEEnit 700, Analytik Jena AG, Germany).

### Extraction of Cd in different chemical forms
According to Zhang et al. [7], six kinds of Cd with different chemical forms were extracted. The extraction sequence was as follows: (1) 80% ethanol (FE-Cd), extracting inorganic Cd and aminophenol Cd; (2) deionized water (FW-Cd), extracting water-soluble Cd from organic acid complexes and $Cd(H_2PO_4)_2$; (3) 1 M NaCl (FNaCl-Cd), extracting Cd integrated with pectate and protein; (4) 2% acetic acid (FHAC-Cd), extracting insoluble $CdHPO_4$, $Cd_3(PO_4)_2$ and other Cd-phosphate complexes; (5) 0.6 M HCl (FHCl-Cd), extracting oxalate acid-bound Cd; and (6) Cd in residues (FC-Cd). Approximately 0.5 g of frozen samples were added to the extraction solution at a ratio of 1:10 (w/v), shaken at $25\,^\circ C$ for 22 h, and centrifuged at $5000 \times g$ for 10 min. The precipitate was resuspended in the same extractive solution twice, shaken at $25\,^\circ C$ for 2 h, and then centrifuged at $5000 \times g$ for 10 min. The supernatant was pooled after three centrifugations and evaporated to 1–2 mL on an electric plate. Each form of Cd was digested with $HNO_3$:$HClO_4$ (4:1, v/v), and the Cd concentration was analyzed by a flame atomic absorption spectrophotometer (ZEEnit 700, Analytik Jena AG, Germany).

### Expression of TaNramp5-a, TaNramp5-b, TaHMA3-a, TaHMA3-b, and TaHMA2
Total RNA was extracted from seedling shoot and root and then used for first-strand cDNA synthesis using the PrimeScript™ RT reagent Kit (TakaRa) in accordance with the manufacturer's protocol. Gene expression was determined using TB green premix Ex Taq™ II (TakaRa). Relative gene expression was calculated by the $2^{-\triangle\triangle Ct}$ method. The expression level of $Cd_5Se_0$, as the normalized mRNA level, was set to 1. The primers for *TaHMA2* were obtained from Tan et al. [19], 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/). The primer sequences are shown in Table S1.

### Statistical analysis

$$Cd\ accumulation = Cd\ concentration \\ \times dry\ matter\ weight$$

$$Cd\ migration\ coefficient = Cd\ concentration\ in\ shoot \\ /Cd\ concentration\ in\ root$$

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

## Results
### Dry matter weight, cd concentration, and accumulation
Cd treatments had significant effects on dry matter weight in shoot and root ($P < 0.01$; Table S2). Cd and Se treatments had significant effects on Cd concentration and accumulation in shoot and root ($P < 0.01$; Table S3); their interaction had significant effects on Cd concentration ($P < 0.01$; Table S3).

Compared with $Cd_5$, $Cd_{25}$ significantly decreased the dry matter weight in the shoot and root. Under Cd stress, Se had no significant effect on the dry matter weight of wheat (Fig. 1).

The Cd concentration in root was higher than that in shoot (Fig. 2a and b). In the $Se_0$ treatment, the Cd concentration in the shoot and root was significantly increased by increasing the Cd stress level; in the $Se_5$ and $Se_{10}$ treatments, the Cd concentration in the root was also significantly increased by increasing the Cd stress level. Compared with $Se_0$, $Se_5$ and $Se_{10}$ significantly decreased the shoot Cd concentration at each Cd stress level, with the degree of decrease ranging from 27.6 to 67.7% (Fig. 2a). Similarly, $Se_5$ and $Se_{10}$ significantly
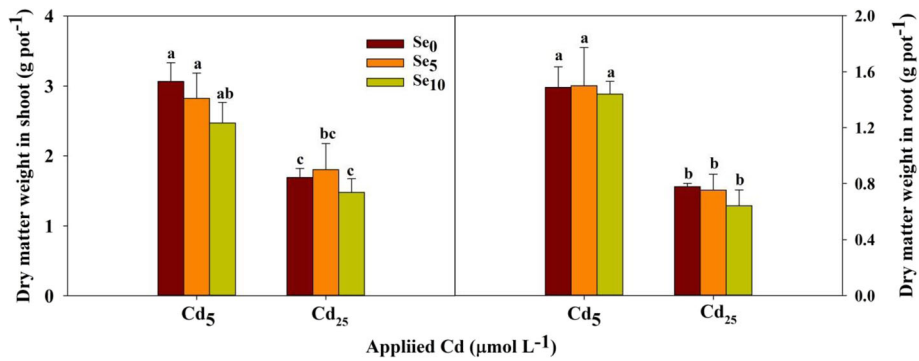
**Fig. 1** Shoot and root dry matter weights of winter wheat (*Triticum aestivum* cv Zhengmai379) seedlings

decreased the root Cd concentration at each Cd stress level, with the degree of decrease ranging from 18.6 to 53.6%, except for the lack of an obvious effect of $Se_5$ on the root Cd concentration at $Cd_5$ (Fig. 2b). The reduction ratio of Cd content in shoot was higher than that in root.

Cd accumulation in root was also higher than that in shoot (Fig. 2c and d). In the $Se_0$ and $Se_5$ treatments, with the increased 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 degree of decrease ranging from 33.3 to 71.6% (Fig. 2c). $Se_{10}$ significantly decreased the root Cd accumulation at each Cd stress level by 46.9 and 61.5% (Fig. 2d).

## Cd migration rate and distribution proportion

Cd and Se treatments had significant effects on the Cd migration rate from root to shoot ($P < 0.01$; Table S3); their interaction had significant effects on the Cd migration rate from root to shoot ($P < 0.05$; Table S3).

Compared with the $Cd_5$ treatment, the Cd migration rate from root to shoot was significantly decreased by the $Cd_{25}$ treatment at $Se_5$ (Fig. 3a). At each Cd stress level, $Se_5$ and $Se_{10}$ significantly reduced the Cd migration rate from root to shoot, with the degree of decrease ranging from 18.8 to 30.3%.

At each Cd stress level, $Se_5$ and $Se_{10}$ significantly reduced the distribution proportion of Cd accumulation in shoot, but $Se_5$ and $Se_{10}$ significantly increased the distribution proportion of Cd accumulation in root (Fig. 3b).
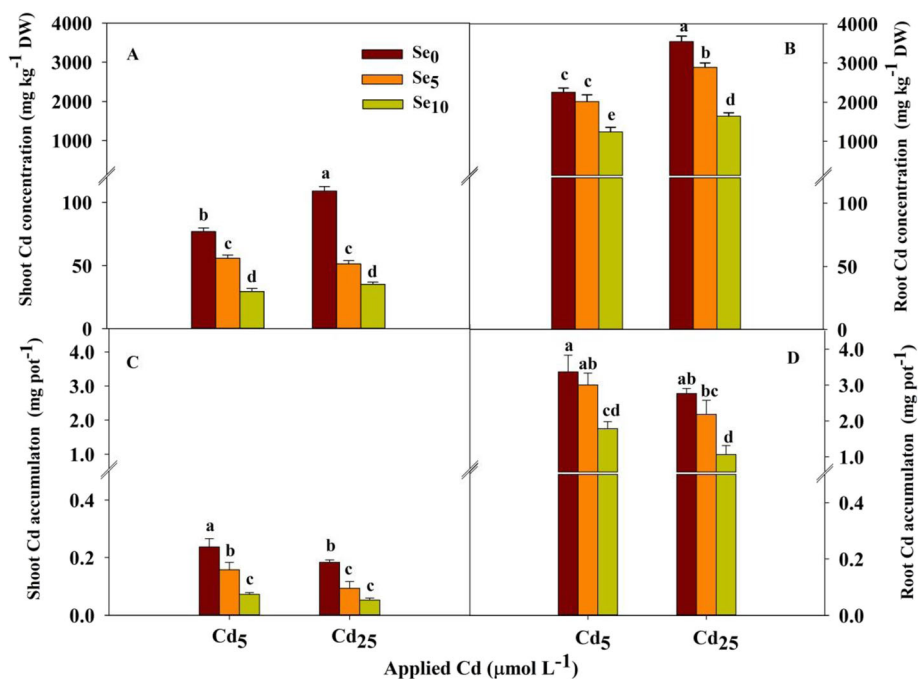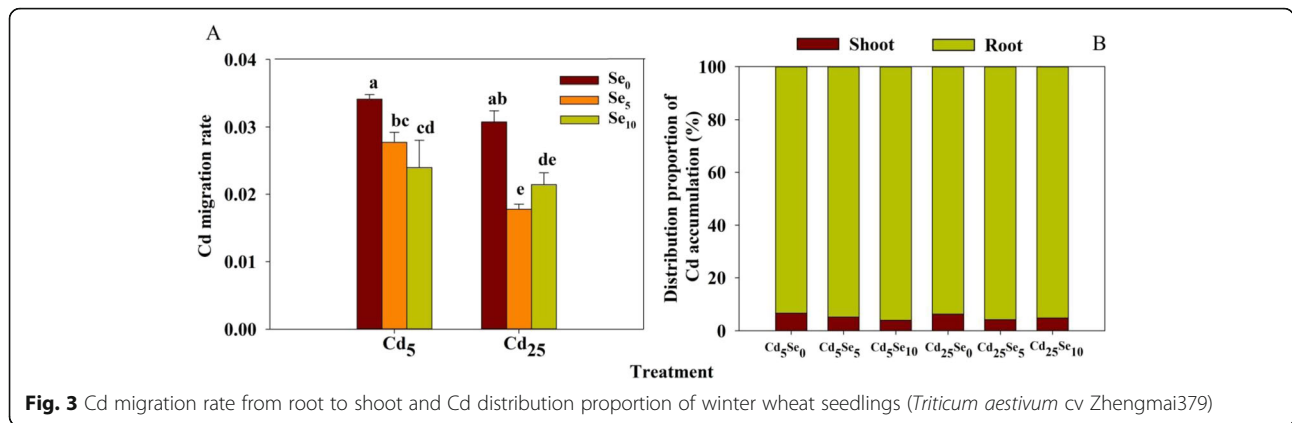


**Fig. 2** Cd concentration and accumulation in shoot (**a** and **c**, respectively) and root (**b** and **d**, respectively) of winter wheat (*Triticum aestivum* cv Zhengmai379) seedlings

Fig. 3 Cd migration rate from root to shoot and Cd distribution proportion of winter wheat seedlings (*Triticum aestivum* cv Zhengmai379)

### Root morphology

Cd and Se treatments and their interaction had significant effects on root length, root total surface area and root volume ($P < 0.01$; Table S4). The Se treatments had significant effects on the average root diameter ($P < 0.01$; Table S4).

The root length, root volume and root surface area were reduced significantly with increasing Cd stress (Fig. 4a, 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 degree of decrease or increase ranging 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 fractionation and distribution

Cd and Se treatments and their interaction had significant effects on the subcellular distribution of Cd in the tissues of wheat seedlings ($P < 0.01$; Table S5).

The Cd concentration in each fraction of shoot and root was significantly increased by increasing the Cd stress level, except for the Cd concentration in the cell wall of shoot at $Se_{10}$, that in the soluble fraction and cell organelles of shoot at $Se_5$ and $Se_{10}$ and that in the cell organelles of root at $Se_{10}$ (Table 1). At $Cd_5$, the Cd concentration in the cell wall, soluble fraction and cell organelles of shoot were significantly decreased by $Se_5$ and $Se_{10}$, with the degree of decrease ranging from 19.0 to 43.2%. $Se_{10}$ significantly decreased the Cd concentration in the soluble fraction and cell organelles of root by 31.3 and 49.3%, respectively. At $Cd_{25}$, $Se_5$ and $Se_{10}$ significantly



Fig. 4 Root morphology parameters of winter wheat seedlings (*Triticum aestivum* cv Zhengmai379)
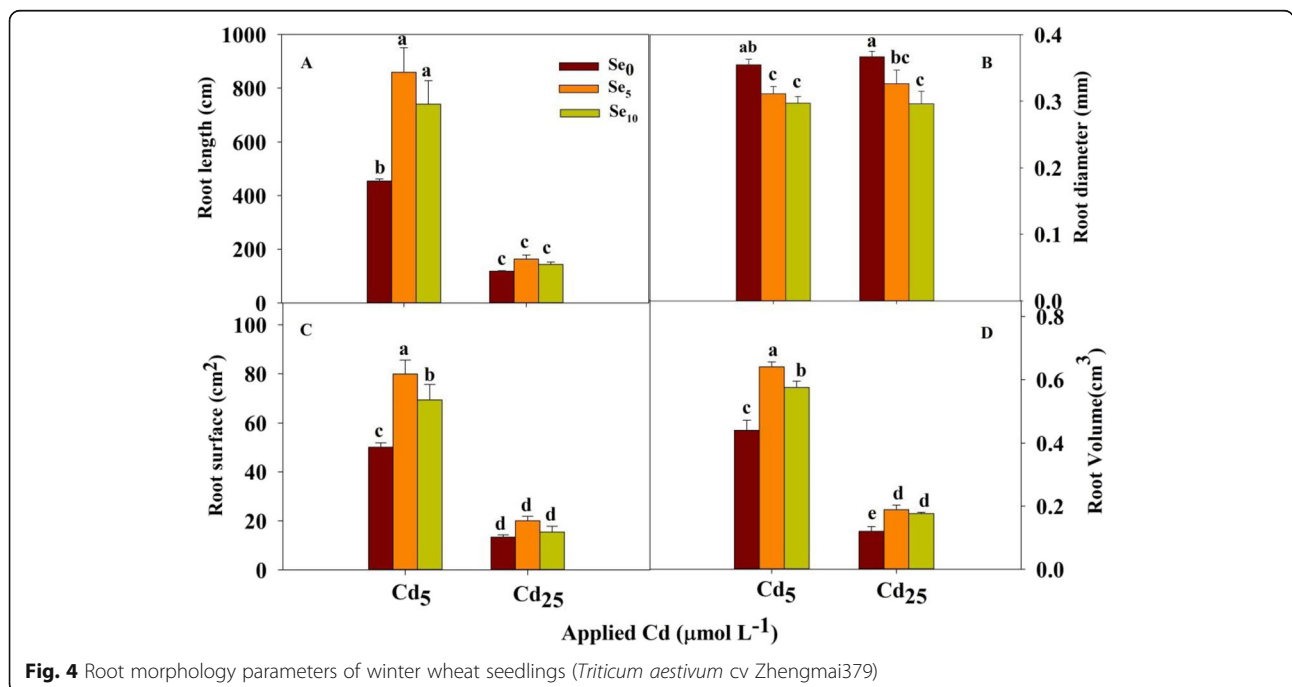
**Table 1** Subcellular fractions of Cd in tissues of winter wheat (*Triticum aestivum* cv Zhengmai379) seedlings grown with low (0 μM), medium (5 μM), or high (10 μM) Se supply under low (5 μM) or high (25 μM) Cd stress for 21 d

| Treatment | | Shoot/(mg·kg$^{-1}$ DW) | | | Root/(mg·kg$^{-1}$ DW) | | |
|---|---|---|---|---|---|---|---|
| Cd (μM·L$^{-1}$) | Se (μM·L$^{-1}$) | Cell wall | Soluble fraction | Cell organelle | Cell wall | Soluble fraction | Cell organelle |
| 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 ± 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)

decreased the Cd concentration in the cell wall, soluble fraction and cell organelles of shoot and root, with the degree of decrease ranging from 17.9 to 65.9%.

In both the shoot and root, the proportion of Cd in the soluble fraction was higher than that in the cell organelles or cell walls (Fig. 5). The Cd proportion in cell organelles of shoot at $Se_0$ and $Se_{10}$, that in the cell wall of shoot at $Se_5$ and $Se_{10}$, and that in the cell wall of root at each Se level was increased by increasing Cd stress level; the Cd proportion in the soluble fraction of shoot and root at each Se level, that in the cell wall of shoot at $Se_0$, and that in cell organelles of root at each Se level was decreased by increasing the Cd stress level. Se application decreased the Cd proportion in the cell organelles of shoot and root at the two Cd levels, with the degree of decrease ranging from 4.65 to 38.0% (Fig. 5a and b). However, Se application increased the Cd proportion in the soluble fraction of shoot and the Cd proportion in the cell wall of root, with the degree of increase ranging from 1.60 to 21.9%. Se application decreased the Cd proportion in the cell wall of shoot at $Cd_5$ but increased its proportion at $Cd_{25}$. $Se_5$ increased but $Se_{10}$ decreased the Cd proportion in the soluble fraction of root.

### Cd chemical forms and distribution
Cd treatments had significant effects on FE-Cd, FNaCl-Cd, FHAC-Cd and FC-Cd concentrations in shoot as

well as the concentrations of FE-Cd, FW-Cd, FNaCl-Cd, FHAC-Cd, FHCl-Cd and FC-Cd in root (*P* < 0.01 or *P* < 0.05; Table S6). Se treatments had significant effects on FE-Cd, FW-Cd, FNaCl-Cd and FHAC-Cd concentrations in shoot as well as the concentrations of FE-Cd, FW-Cd and FNaCl-Cd in root (*P* < 0.01 or *P* < 0.05; Table S6). There was a significant interactive effect of Se and Cd on FE-Cd concentrations in shoot as well as the concentrations of FE-Cd, FW-Cd, FNaCl-Cd and FHAC-Cd in root (*P* < 0.01; Table S6).

At $Se_0$, FE-Cd and FHAC-Cd concentrations in shoot as well as the concentrations of FE-Cd, FW-Cd, FNaCl-Cd, FHAC-Cd and FHCl-Cd in root were significantly increased by increasing the Cd stress level (Table 2). At $Se_5$, with an increased Cd stress level, the FE-Cd concentration in shoot as well as the concentrations of FE-Cd, FW-Cd, FNaCl-Cd and FHAC-Cd in root was markedly increased. At $Se_{10}$, with increased Cd stress level, FE-Cd and FNaCl-Cd concentrations in root were significantly increased; but at $Se_5$ and $Se_{10}$, the FNaCl-Cd concentration in shoot was dramatically reduced by increasing the Cd stress level. At $Cd_5$, $Se_5$ and $Se_{10}$ significantly increased the FE-Cd concentration in root but decreased the FNaCl-Cd in shoot and FW-Cd in root, with the degree of decrease or increase ranging from 25.2 to 60.6% (Table 2). At $Cd_{25}$, $Se_5$ significantly increased the FE-Cd
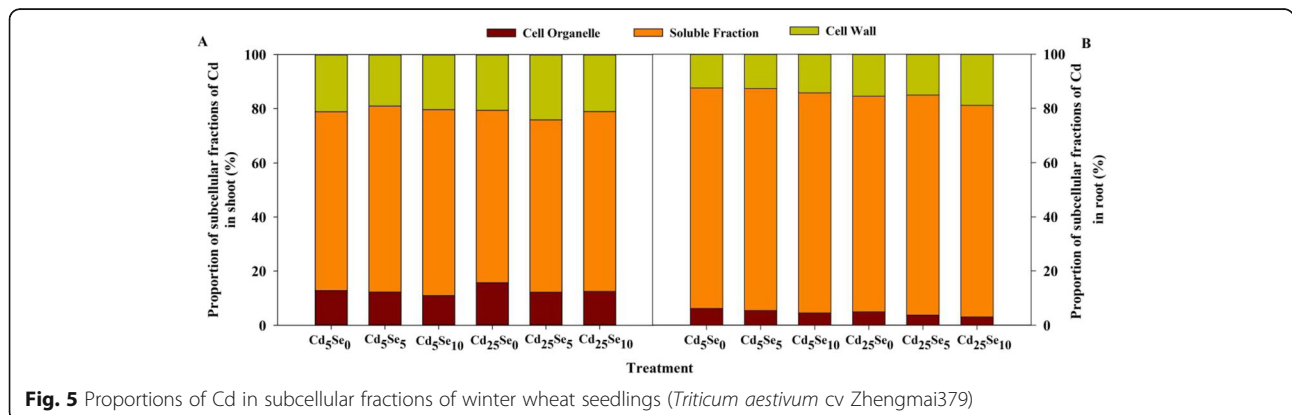


**Fig. 5** Proportions of Cd in subcellular fractions of winter wheat seedlings (*Triticum aestivum* cv Zhengmai379)

**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 | 0.91 ± 0.12bc | 1.35 ± 0.10ab | 14.79 ± 1.23b | 0.74 ± 0.12b | 0.11 ± 0.02a | 0.06 ± 0.01a |
| | | 10 | 0.80 ± 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 | 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 | 0.51 ± 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 | 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 | 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 | 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 | 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 ($P < 0.05$)

concentration in shoot but decreased FHAC-Cd in shoot and FW-Cd and FNaCl-Cd in root. $Se_{10}$ significantly decreased FE-Cd, FW-Cd, FNaCl-Cd and FHAC-Cd in shoot and root, with the degree of decrease or increase ranging from 10.1 to 82.0%.

The Cd proportion in each chemical form in the shoot and root was significantly increased by increasing the Cd stress level, except for FNaCl-Cd in the shoot and root, FW-Cd in root, FHAC-Cd in root at $Se_{10}$, FE-Cd in shoot at $Se_{10}$ and FW-Cd in shoot at $Se_5$ (Fig. 6). In root, $Se_5$ and $Se_{10}$ increased the proportion of FE-Cd, FNaCl-Cd and FHAC-Cd, with the degree of increase ranging from 9.38 to 135%, but $Se_5$ and $Se_{10}$ decreased the proportion of FW-Cd, with the degree of decrease ranging from 14.1 to 43.4%. In shoot, Se increased the proportion of FW-Cd and FE-Cd at $Cd_5$ but decreased the FW-Cd and FE-Cd proportions at $Cd_{25}$, FNaCl-Cd and FHAC-Cd proportions at the two Cd levels, except for

FE-Cd with $Cd_{25}Se_5$ treatment and FNaCl-Cd with $Cd_{25}Se_{10}$ treatment, with the degree of increase or decrease ranging from 1.48 to 107%.

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

Cd and Se treatments and their interaction had significant effects on the transcript levels of *TaNramp5-a*, *TaNramp5-b*, *TaHMA3-a*, *TaHMA3-b*, and *TaHMA2* in shoot and root ($P < 0.05$ or $P < 0.01$; Table S7).

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* with $Cd_5Se_5$, $Cd_{25}Se_5$ and $Cd_{25}Se_{10}$ treatments (Fig. 7). In root, the transcript levels of *TaNramp5-a*, *TaNramp5-b*, *TaHMA3-a* and *TaHMA3-b* were significantly increased by increasing the Cd stress level; increasing Cd stress significantly increased the
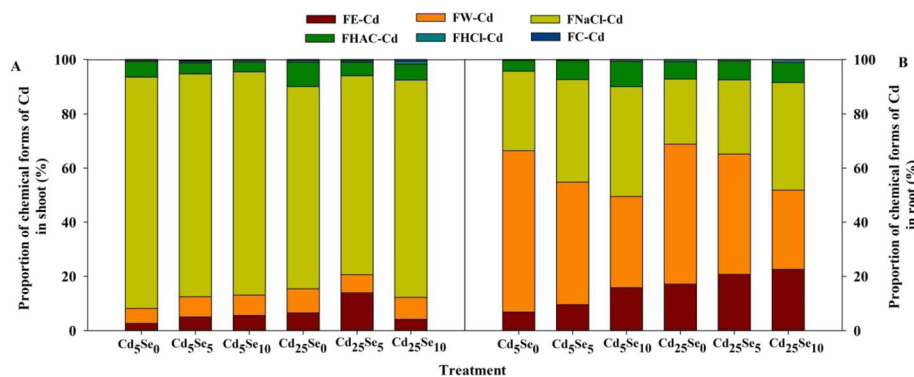


**Fig. 6** Proportions of Cd in chemical forms of winter wheat seedlings (*Triticum aestivum* cv Zhengmai379)
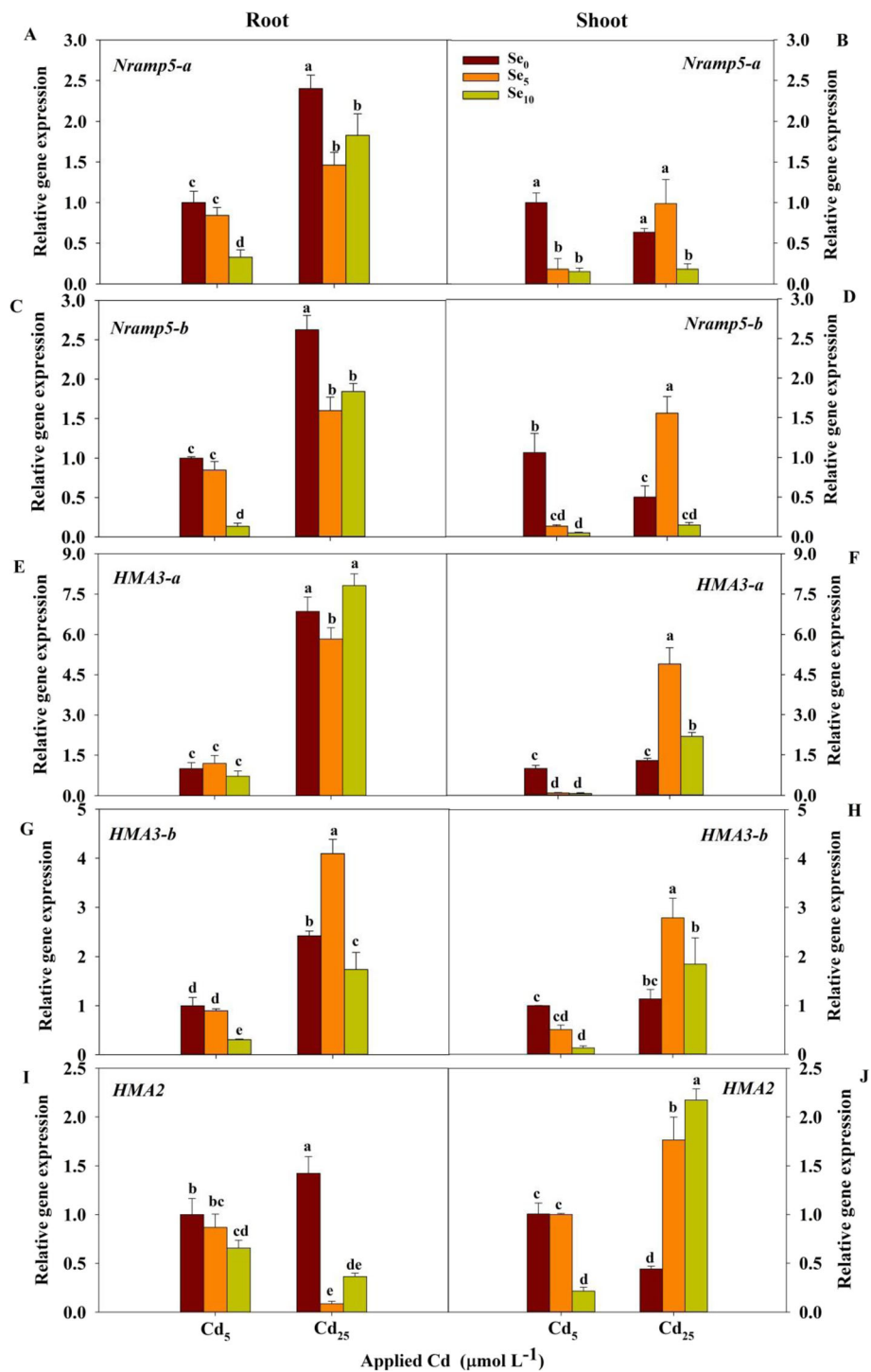
**Fig. 7** The expression of *TaNramp5-a*, *TaNramp5-*b, *TaHMA3-*a, *TaHMA3-*b and *TaHMA2* in winter wheat seedlings (*Triticum aestivum* cv Zhengmai379)

transcript level of *TaHMA2* at $Se_0$ but decreased that at $Se_5$ and $Se_{10}$ (Fig. 7a, 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 transcript levels of the five genes at $Se_5$ as well as the

transcript levels of *TaHMA3-a*, *TaHMA3-b* and *TaHMA2* at $Se_{10}$ (Fig. 7b, 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 $Se_5$ and $Se_{10}$ treatments; $Se_5$ significantly decreased the transcript level of *TaHMA3-a* in root but increased the *TaHMA3-b* transcript level in root and the transcript levels of the five genes in shoot; $Se_{10}$ significantly decreased the *TaHMA3-b* transcript level in root and the *TaNramp5-a* transcript level in shoot but increased the transcript levels of *TaHMA3-a* and *TaHMA2* in shoot.

## Discussion

### Se inhibits Cd absorption by altering root morphology in winter wheat

In our study, the Se supply decreased the Cd concentration and accumulation in both shoot and root (Fig. 2), indicating Se application can inhibit Cd absorption in winter wheat. Huang et al. [20] found that Se application reduced Cd concentration in brown rice by doing a pot experiment, and Lin et al. [21] reported that Se decreases the toxicity and accumulation of Cd in rice by reducing Cd uptake. Plants absorb nutrients mainly through the root system [22]. Many studies have shown that Cd stress can lead to short root length, thick root diameter and a reduced number of lateral root [23]. Our results showed that Se alleviates the toxic effects of Cd on winter wheat root growth, especially under conditions of low Cd stress, showing increased root length, root surface area, and root volume and decreased root diameter with Se application (Fig. 4). However, Ding et al. [24] found that the addition of $0.8\,mg\,L^{-1}$ Se to treatments containing $4\,mg\,L^{-1}$ Cd increased the length, surface area, volume, and average diameter of root in rice. Root morphology has a great influence on the absorption of minerals [5]. Fine root are the most active part of the root system for mineral absorption [24, 25]. Nazar et al. [26] noted that plant nutrients, such as iron (Fe), manganese (Mn), 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 by altering the distribution of Cd in subcellular fractions and by altering Cd chemical forms in winter wheat tissues

Our study suggested $Se_5$ and $Se_{10}$ significantly decreased Cd migration rate from root to shoot, the distribution proportion of Cd accumulation in shoot, and the Cd concentration in the cell wall, soluble fraction and cell organelles of shoot and root (Table 1 and Fig. 3). The decreased Cd concentration in subcellular fractions was due to the decreased Cd concentration in winter wheat following Se application. These results also suggested that most of the Cd accumulated in the soluble fraction, followed by the cell wall fraction (Table 1 and Fig. 5). Our results are consistent with the results of Li et al. [27], 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 the soluble fraction and cell wall is easily chelated and fixed by organic substances, so it is difficult to transfer to other fractions [28]. Li et al. [29] 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, vacuoles (involved in the soluble fraction) are considered to accumulate the greatest amount of Cd and are the place where waste and byproducts accumulate [30]. Heavy metals can be separated in vacuoles by binding with various proteins, organic acids and organic bases [31]. In our study, Se application enhanced Cd accumulation in the soluble fraction of shoot (Fig. 5a), indicating that Se use can inhibit Cd migration to other organs, thus alleviating Cd toxicity. The cell wall fraction can bind Cd ions and reduce transport to other parts of the plant, which is the first barrier to protect protoplasts from Cd toxicity [32]. The Cd proportion in the cell wall of root was increased by $Se_5$ and $Se_{10}$ (Fig. 5b), suggesting that Se application enhanced Cd accumulation in root to inhibit Cd transport from root to shoot.

Different chemical forms of Cd have distinct migration capacities. 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 abilities and cause greater harm to plant cells [7]. Some studies have shown that FNaCl-Cd plays an important role in the alleviation of Cd toxicity [18, 33]. In our study, Cd was mainly integrated with pectate and protein (FNaCl-Cd) in the shoot and existed in the form of FW-Cd and FNaCl-Cd in the root (Table 2 and Fig. 6). This result indicates that Cd easily migrates from root to shoot in the water-soluble form, but the toxicity of Cd can also be alleviated by converting Cd into undissolved pectate and protein-bound forms. Qiu et al. [34] found that the majority of Cd in both the root and shoot of cabbage was extracted by 1 M NaCl. Some specific polarcompounds contain hydroxyl or carboxyl groups, which can combine with Cd to form a nontoxic complex [18]. Se treatment 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 reduces the mobility of Cd from root to shoot by promoting the transformation of Cd from the active form to the inactive form in root. The total proportion of active Cd

(FE-Cd and FW-Cd) in shoot was decreased by introducing high levels of Se ($Se_{10}$) at $Cd_{25}$, suggesting that a high level of Se can inhibit the mobility of Cd in shoot at a high Cd stress level.

### Downregulation 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 [35]. *Nramp5* is a member of the Nramp family, located on the plasma membrane of plant root [35]. In our study, the expression of *TaNramp5-a* and *TaNramp5-b* was found in both root and shoot and was significantly increased with increasing Cd concentrations (Fig. 7a, b, c and d), suggesting that Nramp5 might be involved in the absorption and transport of Cd in wheat plants. This finding is in agreement with the results of Ma et al. [36], who showed that the expression of *OsNramp5* was significantly increased at increased Cd concentrations. Tang et al. [37] and Sasaki et al. [35] observed that knockout of *OsNramp5* can significantly reduce the Cd concentration in the root and shoot of rice. In our study, Se use significantly decreased the expression of *TaNramp5-a* and *TaNramp5-b* in shoot at low Cd stress levels (Fig. 7b and d), indicating that Se might inhibit the remobilization of Cd in shoot. In addition, Se treatment significantly decreased the expression of *TaNramp5-a* and *TaNramp5-b* in root (Fig. 7a and c), indicating that the downregulation of *TaNramp5-a* and *TaNramp5-b* by Se may help to decrease Cd uptake in wheat. Cui et al. [38] found that Se pretreatment decreased the expression of *OsNramp5,* thus inhibiting Cd uptake.

Heavy metal ATPases (HMAs) are 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. 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 [39]. Sasaki et al. [40] 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 was significantly increased with increasing Cd concentrations (Fig. 7e, f, g and h), suggesting that HMA3 might be responsible for the transport of Cd in wheat plants. Se treatment downregulated the expression of *HMA3* in shoot at $Cd_5$ but upregulated expression at $Cd_{25}$ (Fig. 7f and h), also indicating that Se can inhibit the remobilization of Cd in shoot by enhancing the sequestration of Cd into vacuoles when Cd stress levels are high. Cui et al. [38] showed that Se pretreatment activated the expression of *OsHMA3,* thus enhancing the transport of Cd into vacuoles.

*HMA2* (heavy metal ATPase2), which is homologous with *HMA3*, belongs to the heavy metal ATPase family. *HMA2* plays a role in the loading of Cd and Zn into xylem and is involved in the root-to-shoot translocation of Cd and Zn [19]. Our results showed that the expression of *TaHMA2* was found in both root and shoot and that it significantly increased with increasing Cd concentrations (Fig. 7i and j). This result suggested that HMA2 might be involved in the transport of Cd in wheat plants. This conclusion is consistent with the results of Tan et al. [19], who showed that the overexpression of HMA2 in wheat and rice increased the root-shoot translocation of Zn/Cd. A recent report showed that lanthanum decreased Cd accumulation in wheat, which may be related to *TaHMA2* downregulation [41]. In our study, Se treatment significantly decreased the expression of *TaHMA2* in root, indicating that the downregulation of *TaHMA2* by Se might contribute to the inhibition of Cd root-to-shoot translocation and the final decrease in Cd accumulation in the shoot of winter wheat. The expression of *TaHMA2* in shoot was significantly increased by Se treatment at $Cd_{25}$ (Fig. 7j), suggesting that Se may promote the remobilization of Cd in shoot by upregulating the expression of *TaHMA2* when the Cd stress level is high. In our studies, chemical forms and transporter processes were affected by the Cd concentration or accumulation. The downregulation of *TaNramp5-a* and *TaNramp5-b* decreased Cd uptake by wheat root and then decreased the Cd content in wheat. The downregulation of *TaHMA2* and the proportion of active Cd in root reduced the migration of Cd to shoot and the distribution of Cd in shoot.

## Conclusions

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

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-020-02763-z.

**Additional file 1.**

**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

## Ethics approval and consent to participate
Not applicable.

## Consent for publication
Not applicable.

## Competing interests
The authors declare that they have no competing interests.

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