**RESEARCH ARTICLE** 



### NPK-N application limits grain cadmium concentration of wheat via promoting Cd export during grain filling

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### Abstract

*Aims* Wheat easily absorbs Cadmium (Cd) from soils and accumulates in its grains. Nitrogen (N) fertilizer management has been used to limit grain Cd concentrations in wheat, but the effects of compound fertilizers (NPK-N) are unclear.

*Methods* Here, three years' of field experiments were conducted to evaluate the effects of NPK-N on grain Cd concentration and yield of 50 wheat cultivars. A high-Cd accumulating cultivar Chuanmai 68 (CM68) and a low-Cd accumulating Mianmai 902 (MM902)

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Y. He · X. Chen · Y. Ren (⊠) Mianyang Academy of Agricultural Science/Crop Characteristic Resources Creation and Utilization Key Laboratory of Sichuan Province, Mianyang 621000, Sichuan, China e-mail: rywheat@126.com were further used to reveal the physiological and molecular mechanisms of the Cd transport pathway. *Results* The results showed that NPK-N application significantly reduced grain Cd accumulation in all 50 wheat cultivars compared to amide N fertilize (urea-N) application. MM902 showed lower Cd uptake before anthesis, higher Cd export from plant during grain filling, and lower grain Cd concentration than CM68. Meanwhile, NPK-N application significantly reduced grain Cd concentration in CM68 (39.70%) and MM902 (35.12%), as well as Cd uptake, but promoted Cd export compared to urea-N. Besides, the expression levels of TaIRT1 (Iron Regulated Transporter 1) and TaCCX2 (Cation/Ca Exchanger 2) were associated with Cd uptake and export, respectively. Overexpression of TaIRT1 increased Cd uptake and grain Cd concentration in rice.

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L. Sha · H. Zhang College of Grassland Science and Technology, Sichuan Agricultural University, Wenjiang 611130, Sichuan, China *Conclusions* Our study showed that NPK-N application can limit grain Cd concentration and increase grain yield of wheat. The different grain Cd concentrations between CM68 and MM902 are mainly results from the different Cd uptake before anthesis and Cd export during grain filling. The results provide a theoretical basis for safe and high-quality wheat production by NPK-N application.

Keywords Wheat  $\cdot$  Cadmium  $\cdot$  Nitrogen  $\cdot$  Yield  $\cdot$  Uptake

#### Introduction

Cadmium (Cd), a toxic heavy metal, is non-biodegradable and harmful to all living organisms. Many arable soils around the world are contaminated with Cd released from industrial and agricultural processes (The Ministry of Environmental Protection and Ministry of Land and Resources, 2014; Sun et al. 2023). For example, there are approximately 278,600 ha of Cd-contaminated arable soils in China (Ata-UI-Karim et al. 2020). Cereal crops grown on Cd-contaminated soils accumulate high concentrations of Cd in their edible parts (Yan et al. 2018), posing potential risks to human health (Adler et al. 2023).

Wheat is a staple food for nearly half of the global population, and easily absorbs Cd from soils and accumulates in its grains. To protect the food safety of wheat production, the Codex Alimentarius Commission set the safety standard for Cd concentration in wheat grains at less than 0.20 µg/g, and in China, the National Standard for Food Safety Limits of Contaminants in Food (GB 2762–2022) set it as 0.10 µg/g (Codex Alimentarius Commission 1995; National Health Commission of the People's Republic of China, State Administration for Market Regulation, 2022). However, with the increasing of Cd-contaminated arable soils of wheat-producing areas, incidences of wheat grain accumulating Cd exceeding national and international standards have been increasing (Ran et al. 2016; Cai et al. 2019; Li et al. 2019). Therefore, reducing grain Cd concentration in wheat is an important goal to ensure food safety and human health.

Grain Cd concentration in wheat is mainly mediated by root Cd uptake, root-to-shoot Cd translocation, and shoot Cd distribution and redistribution (Kubo et al. 2016; Liang et al. 2017; Yan et al. 2018, 2019; Cheng et al. 2021). These processes are mainly controlled by genetic factors such as metal transporter genes (Cheng et al. 2024), and also mediated by agricultural strategies such as nitrogen (N) fertilization management (Yang et al. 2020; Cheng et al. 2021; Li et al. 2022a).

Nitrogen is essential for plant growth and development. It is involved in biomolecular synthesis, cellular metabolism, signal transduction and organ morphogenesis, and then mediates biomass and yield (Yang et al. 2020). N fertilizers include different N chemical forms, such as amide N fertilizer (urea-N), nitrate N fertilizer (NO<sub>3</sub><sup>-</sup>-N), and ammonium N fertilizer (NH<sub>4</sub><sup>+</sup>-N) (Yang et al. 2016). Urea-N,  $NO_3^{-}-N$ , and NH<sub>4</sub><sup>+</sup>-N not only differentially affect soil properties such as pH and Cd bioavailability, but also change plant cell membrane permeability, expressions of metal transport genes, and polysaccharide composition and concentration of the cell wall. Thus, they differentially alter Cd uptake, translocation, and accumulation (Cheng et al. 2016, 2018, 2020; 2021; Yang et al. 2016, 2020). However, the different effects depend on the wheat species and cultivar. For example, application of urea-N, ammonium nitrate, or calcium ammonium nitrate increased grain Cd concentration in winter wheat (Svecnjak et al. 2013), durum wheat (Gray et al. 2002; Mitchell et al. 2000; Perilli et al. 2010), and spring milling wheat (Li et al. 2011); while, application of NO<sub>3</sub><sup>-</sup>-N decreased it in another durum wheat cultivar (Tosun et al. 2023). Our previous study found that NH4+-N application increased grain Cd concentration in a low-Cd accumulating winter wheat cultivar but did not affect that in a high-Cd accumulating cultivar (Cheng et al. 2021). Currently, a new form of N fertilizer, NPK-N compound (NPK-N), is preferentially used as the basic fertilizer for wheat production (Guidelines for Scientific Fertilization of Major Crops in Sichuan Province in 2021–2025), rather than urea-N (a traditional N fertilizer) (Li et al. 2022b). However, its effects on grain Cd concentrations in wheat and its relative physiological mechanisms are poorly understood.

Cd transport in plants is usually mediated by essential metal transporters, such as natural resistance-associated macrophage proteins (NRAMPs), heavy metal P-type ATPases (HMAs), cation/Ca exchangers (CCXs) and ZRT/IRT-related proteins (ZIPs) (Cheng et al. 2024; Huang et al. 2024). In wheat, our previous results have shown that several metal transporters, such as TpNRAMP3, TpNRAMP5, TpIRT1 and TpCCX2, can transport Cd (Peng et al. 2018a, 2018b; Jiang et al. 2021; Cheng et al. 2024). In addition, the expression of metal transporter genes, such as *IRT1*, *NRAMP5*, *NRAMP1*, *CCX2*, *ZIP5*, and *HMA2*, can be regulated by NO<sub>3</sub><sup>-</sup>-N or NH<sub>4</sub><sup>-</sup>-N, resulting in altered Cd uptake and accumulation in wheat, rice and/ or Arabidopsis (Yang et al. 2016, 2020; Wu et al. 2018; Cheng et al. 2020, 2021). However, it is unknown whether and how NPK-N application regulates the expression of metal transporter genes in wheat, thereby affecting Cd uptake and accumulation.

To investigate the effects of NPK-N application on grain Cd concentration in wheat, we firstly performed three-year field experiments with applications of NPK-N and urea-N to evaluate the effects of NPK-N application on grain Cd concentration using 50 wheat cultivars. Second, a low-Cd accumulating wheat cultivar and a high-Cd accumulating wheat cultivar were used to further analyze plant growth, yield, grain Cd concentration, Cd uptake, root-to-shoot Cd translocation, shoot Cd distribution and redistribution, and expression levels of metal transporter genes (such as TaIRT1) under urea-N and NPK-N applications. Finally, we overexpressed a metal transporter gene whose expression was regulated by NPK-N application in rice to confirm its effect on grain Cd concentration. These results provide theoretical guidance and technical support for high-yield and high-quality wheat production.

### Materials and methods

#### Plant materials and field experiment

The experiment was conducted during the wheat growing seasons from 2019 to 2023 at the different experimental field of Sichuan Agricultural University, Wenjiang, China. The soil Cd concentrations were 0.30 mg/kg (pH=6.05), 0.23 (pH=6.20), 0.25 mg/kg (pH=6.25) and 0.28 mg/kg (pH=6.01) in the wheat growing seasons of 2019–2020, 2020–2021, 2021–2022 and 2022–2023, respectively. The schematic diagram of the field experiment are shown in Fig. S1.

Experiment 1: Evaluation of 50 wheat cultivars for grain Cd concentration and grain yield under NPK-N and urea-N applications

Fifty Chinese common wheat (Triticum aestivum L.) cultivars were collected from the Southwest, Huanghuaihai, middle and lower reaches of the Yangtze River and Northwest wheat regions. They were planted during the 2019-2020 wheat growing season using a randomized complete block design with three replicates. For each replicate, each cultivar was planted in three plots, each plot consisting of 50 cultivars (10 rows for each cultivar), each 1.5 m long and 30 cm apart. Cultivation and field management were applied according to local practices. The "Guidelines for Scientific Fertilization of Major Crops in Sichuan Province in 2021-2025" recommended 90 kg/ha of compound fertilizer (N:P:K=15:15:15) as the base fertilizer before sowing and 90 kg/ha of urea-N as the follow-up fertilizer at the tillering stage. In this study, two treatments were performed to investigate the effects of NPK-N on grain Cd concentration: (1) urea-N, with urea-N (containing 90 kg/ha of N), P2O5 (90 kg/ha) and K2O (90 kg/ha) as basal fertilizer, and urea-N (containing 90 kg/ha of N) as a follow-up fertilizer; (2) NPK-N, with compound fertilizer at 600 kg/ha (N, P2O5, and K<sub>2</sub>O content of 90 kg/ha, respectively) and urea-N (containing N content of 90 kg/ha) as a follow-up fertilizer. At maturity, grains sampled of each cultivar were collected to measure Cd concentration and yield.

In addition, 50 wheat cultivars were also planted during the 2020–2021 and 2021–2022 wheat growing seasons to validate the effect of NPK-N application on grain Cd concentration. The experiment was conducted and managed as described above. At maturity, grains sampled from these cultivars were used to measure Cd concentration.

Experiment 2: Analysis of the effects of urea-N and NPK-N application on the Cd transport pathway in high- and low-Cd accumulating wheat cultivars

Based on the grain Cd concentration of 50 wheat cultivars, a high-Cd accumulating cultivar (H-Cd) and a low-Cd accumulating cultivar (L-Cd) were used to further analyze the effects of urea-N and NPK-N on grain Cd concentration and yield, as well as the Cd transport pathway. The experiment was conducted during the 2022–2023 wheat growing season using a randomized block design with three replicates as described above. The soil properties of the treatments are shown in Table S1 (NPK-N application increased soil pH and decreased available Cd concentration than urea-N application). At anthesis and maturity, 30 plants (three plants per row) were randomly harvested from each plot and used to investigate agronomic traits. The plant parts were separated into grains, glumes, rachises, internode I, flag leaves, node I, lower internodes, lower leaves, lower nodes, and roots. All plant samples were dried at 105  $^{\circ}$ C for 30 min and at 80  $^{\circ}$ C for 3 d and then weighed and ground to determine Cd concentration.

Investigation of agronomic traits and biomass

At maturity, the agronomic traits, including tiller number, plant height, flag leaf length, flag leaf width, flag leaf area, spike length, spikelet number, grain length, grain width, thousand grain weight, grain number per plant, and grain yield per plant of H-Cd and L-Cd were measured. The dry weights of grains, glume rachises, internode I, flag leaves, node I, lower internodes, lower leaves, lower nodes, roots, and the whole plant were determined. Determination of Cd and other metal concentrations

Cd and other metal concentrations were determined using the method described by Cheng et al. (2018, 2020). In brief, each sample (0.20 g powder) was digested with a mixed acid solution [HNO<sub>3</sub>/HClO<sub>4</sub> (v/v=4/1)] at 280 °C for 4 h, sequentially diluted to 25 mL with deionized water, and filtered through filter paper. Metal concentrations were determined using an inductively coupled plasma mass spectrometer (ICP-MS, 7900; Agilent, Palo Alto, CA, USA). A certified reference material (wheat [GBW 10011]; National Research Center for Standards, China) was used for quality assurance.

Calculation of Cd content, uptake, translocation, distribution, redistribution, and continuous absorption/export

Tissue Cd content, whole-plant Cd uptake, rootto-shoot Cd translocation, tissue Cd distribution at anthesis and maturity, whole-plant continuous Cd absorption/export and shoot Cd redistribution during grain filling were calculated using the methods of Kubo et al. (2016) and Cheng et al. (2021, 2024).

Tissue Cd content  $(\mu g)$  = Tissue Cd concentration  $(\mu g/g) \times$  tissue dry weight (g) Whole – plant Cd accumulation = whole – plant Cd content  $(\mu g) = \sum$  Cd content in all tissues Whole – plant Cd uptake = whole – plant Cd concentration  $(\mu g/g)$  = whole – plant Cd content  $(\mu g)$ / Whole – plant dry weight (g) Root – to – shoot Cd translocation = Shoot Cd content  $(\mu g)$  / Whole – plant Cd content  $(\mu g)$ Shoot Cd distribution factor = Tissue Cd content  $(\mu g)$  / Shoot Cd content  $(\mu g)$ Shoot Cd redistribution = Tissue Cd distribution factor at maturity – Tissue Cd distribution factor at anthesis Continuous Cd absorption/export  $(\mu g)$  = whole – plant Cd content at maturity  $(\mu g)$  – whole – plant Cd content at anthesis  $(\mu g)$ ; positive and negative values indicated Cd export and absorption, respectively

### Total RNA extraction and RT-qPCR

Roots, node I, rachises and glumes of H-Cd and L-Cd were collected at anthesis and/or grain filling. Total RNA isolation and cDNA synthesis were performed as described previously (Cheng et al. 2024). The expression levels of metal transporter genes (*TaNRAMP1*,

*TaNRAMP2*, *TaNRAMP5*, *TaZIP5*, *TaCCX2*, *TaN-RAMP3*, *TaHMA2* and *TaLCT1*) were normalized using RT-qPCR as described by Chai et al. (2022). The specific primers used are listed in Table S2, and *TaACTIN* and *TaGAPDH* were used as reference genes (Wang et al. 2015; Jiang et al. 2021).

### Overexpression of TaIRT1 in rice

The CDS of TaYSL15 cloned from L-Cd was subcloned into the overexpression vector BGV002 under the control of the 35S promoter. The recombinant vector was transformed into rice cultivar Zhonghua11 using Agrobacterium-mediated transformation as described by Han et al. (2023). The expression level of TaIRT1 in the overexpression lines was normalized using OsAC-TIN as a reference gene (Yang et al. 2011). Zhonghua11 and TaIRT1-overexpressing lines were grown in the greenhouse at Sichuan Agricultural University. At maturity, agronomic traits (plant height, tiller number, branches number, grain length, grain width, thousand grain weight, grain number per plant and grain yield per plant), tissue Cd concentration, Cd uptake, root-toshoot Cd translocation and shoot-to-grain Cd distribution were calculated as described above.

### Statistical analyses

All data were analyzed using one-way analysis of variance (ANOVA). Duncan's test was used to compare differences at a significance level of  $P \le 0.05$  by using SPSS (version 20.0). An independent sample t test was used to test for significant differences between

two samples. Pearson's correlation was used to analyze the correlations between treatments (P < 0.05, P < 0.01). All graphs were constructed using Sigma-Plot (version 12.0).

### Results

### NPK-N application reduced the grain Cd concentration of 50 wheat cultivars

Grain Cd concentrations and yields of 50 wheat cultivars grown on Cd-contaminated soils under urea-N and NPK-N applications during the 2019–2020 wheat growing season were investigated. Under urea-N application, all cultivars accumulated more than 0.10 mg/kg of Cd in their grains, varying from 0.13 to 0.39 mg/kg with an average of 0.22 mg/kg; and thirtythree cultivars accumulated grain Cd concentrations equal to or greater than 0.20 mg/kg (Fig. 1). However, NPK-N application significantly reduced the average of grain Cd concentration to 0.16 mg/kg with a range from 0.08 to 0.21 mg/kg (Fig. 1). Similar results were observed in other two-year NPK-N application treatments, with grain Cd concentrations of 50 cultivars ranging from 0.02 to 0.16 mg/kg (with mean of



Fig. 1 Grain Cd concentration of 50 wheat cultivars. Grain Cd concentration of 50 wheat cultivars at 2019–2020, 2020–2021 and 2021–2022 wheat growing season. "\*" indicates significant difference between urea-N and three years' of NPK-N application

0.09 mg/kg) and from 0.05 to 0.18 mg/kg (with mean of 0.12 mg/kg), respectively (Fig. 1). Meanwhile, the grain yield per plant of 50 cultivars varied from 4.26 to 21.11 g with a mean of 11.95 g under urea-N application; but NPK-N application significantly promoted the average of grain yield per plant to 16.33 g with a variation from 5.35 to 29.12 g (Fig. S2). These results indicated that NPK-N application not only reduced grain Cd concentration, but also increased grain yield.

Among these 50 cultivars, only Mianmai 902 (MM902) and Aikang 58 showed low grain Cd concentrations, less than 0.10 mg/kg over three years of NPK-N application (Fig. 1), defined as low-Cd accumulators.

Fig. 2 Agronomic and yield traits under urea-N and NPK-N at maturity. A Plant height; B spike length; C tiller number; D flag leaves length; E flag leaves width; F flag leaves area; G spikelet number; H thousand grain weight; I grain length; J grain width; K grain number per plant; L grain yield per plant. Different letters indicate significance level at P < 0.05by the Duncan's test Nineteen cultivars, such as Chuanmai 68 (CM68), accumulated high concentrations of Cd in grains, equal to or more than 0.10 mg/kg in all NPK-N application experiments, recommended as high-Cd accumulators (Fig. 1). The grain Cd concentrations of the other 29 cultivars were unstable over the three-year experiments, which were influenced by environmental factors (Fig. 1).

### Agronomic and yield traits of high- and low-Cd accumulators under urea-N and NPK-N applications

To reveal the mechanisms that NPK-N application reduced grain Cd concentrations, MM902 (a low-Cd



accumulator) and CM68 (a high-Cd accumulator) were further grown under urea-N and NPK-N applications to compare growth and Cd transport pathways.

For agronomic traits, plant height of MM902 was significantly shorter than that of CM68 under urea-N application (Fig. 2A); the opposite result was found for spike length (Fig. 2B); and other traits including tiller number, flag leaf length, flag leaf width, flag leaf area and spikelet number were not different between CM68 and MM902 (Fig. 2C-G). Under NPK-N application, spike length of MM902 was higher than that of CM68, but there was no difference in other agronomic traits (Fig. 2A-G). Compared with urea-N application, NPK-N application dramatically increased the plant height of MM902 and the spike length of CM68 (Fig. 2A-B).

For yield traits, urea-N application did not cause significant differences in thousand grain weight, grain length and grain width between CM68 and MM902 (Fig. 2H-J), but the grain number and yield per plant of MM902 were higher than those of CM68 (Fig. 2K-L). Under NPK-N application, the thousand grain weight was significantly higher in MM902 than in CM68 (Fig. 2H). However, there was no significant difference in grain length, grain width, grain number and yield per plant between two cultivars (Fig. 2I-L). Compared with urea-N application, NPK-N application increased thousand grain weight (20.37%) of MM902, and grain number (106.83%) and grain yield (101.97%) per plant of CM68 (Fig. 2H, K, L). These results indicated that NPK-N application promoted the growth and development of CM68 and MM902 more than urea-N application.

Dry weight of high- and low-Cd accumulators under urea-N and NPK-N applications

At maturity, the dry weights of grains, glumes, rachises, lower leaves, shoots and whole plants were higher in MM902 than in CM68 under urea-N application (Table 1); and no significant difference was observed under NPK-N application (Table 1). NPK-N application significantly increased the dry weight of grains and other tissues (46.67%–106.61%, except for internode I, lower nodes, and roots), shoots (79.49%), and whole plant (77.43%) of CM68, but did not alter the dry weight of MM902 compared to urea-N application (Table 1).

Tissue Cd concentration and content of high- and low-Cd accumulators under urea-N and NPK-N applications

At maturity, grain Cd concentrations of MM902 and CM68 under urea-N application were 0.12 and 0.23 µg/g, respectively. Compared with urea-N application, NPK-N application effectively and significantly reduced grain Cd concentrations of MM902 to 0.08 mg/kg (35.12%) and CM68 to 0.14 mg/kg (39.70%) (Fig. 3A). However, NPK-N application slightly decreased grain Mn and Zn concentrations but not Fe and Cu concentrations of CM68, and it did not affect grain Mn, Zn, Fe and Cu concentrations of MM902 (Table S3). These results indicated that NPK-N application only decreased grain Cd concentration in both two cultivars. Subsequently, we focus

Tissue dry weight (g)	Urea-N		NPK-N	
	CM68	MM902	CM68	MM902
Grains	$8.62 \pm 0.96b$	20.30±3.15a	17.81 ± 2.68a	21.73±0.95a
Glumes	$4.35 \pm 0.89b$	7.79±4.35a	$7.03 \pm 2.50a$	$7.10 \pm 2.85a$
Rachises	$0.74 \pm 0.12b$	$1.39 \pm 0.62a$	$1.35 \pm 0.46a$	$1.36 \pm 0.32a$
Internode I	1.59±0.11a	$2.41 \pm 0.81a$	$1.95 \pm 0.54a$	$2.53 \pm 0.56a$
Flag leaves	$1.54 \pm 0.35b$	$2.26 \pm 1.07$ ab	$3.09 \pm 1.33a$	2.61±0.31a
Node I	$0.30 \pm 0.02b$	0.37±0.13ab	$0.44 \pm 0.34a$	$0.50 \pm 0.14a$
Lower internodes	$4.28 \pm 0.14b$	$4.07 \pm 0.97$ b	$7.00 \pm 3.09a$	$6.00 \pm 1.82a$
Lower leaves	$2.86 \pm 0.34b$	4.31 ± 1.28a	$4.95 \pm 2.41a$	4.65±0.99a
Lower nodes	$0.83 \pm 0.10a$	1.16±0.37a	$1.45 \pm 0.68a$	$1.71 \pm 0.40a$
Roots	$0.76 \pm 0.17a$	0.67 ± 0.17a	$0.83 \pm 0.26a$	$0.87 \pm 0.41a$
Shoots	$25.11 \pm 1.82b$	44.06 ± 6.74a	45.07±5.11a	48.19±8.03a
Whole-plant	$25.87 \pm 2.28 \mathrm{b}$	44.73 <u>+</u> 8.55a	$45.90 \pm 6.71a$	$49.06 \pm 8.03a$

**Table 1** Dry weights oftissue and whole-plant atmaturity



Fig. 3 Tissue Cd concentration at maturity. A-J Cd concentrations in grains (A), glumes (B), rachises (C), internode I (D), flag leaves (E), node I (F), lower internodes (G), lower leaves

(H), lower nodes (I), and roots (J) of CM68 and MM902 at maturity under urea-N and NPK applications. Different letters indicate significance level at P < 0.05 by the Duncan's test

on Cd concentration and content in other tissues to elucidate the Cd transport pathway.

Further investigation showed that Cd concentrations of glumes, rachises, internode I, flag leaves, node I, lower internodes, lower leaves, lower nodes, and roots were significantly lower in MM902 than in CM68 at maturity under both urea-N and NPK-N applications (Fig. 3B-J). Meanwhile, NPK-N application also significantly reduced Cd concentrations of all tissues in both MM902 and CM98 by 17.04–56.36% compared to urea-N application (Fig. 3A-J). However, different results were observed at anthesis (Fig. S2A-I). For example, the Cd concentrations of node I, lower nodes, and roots were higher in MM902 than in CM68 under NPK-N application (Fig. S2E, H, I). Compared to urea-N application, NPK-N application increased Cd concentrations of glumes, internode I, flag leaves, lower internodes,

Table 2 Tissue and whole-plant Cd contents of CM68 and MM902 at anthesis and maturity

Tissue Cd	At anthesis				At maturity			
content (µg)	urea-N		NPK-N		urea-N		NPK-N	
	CM68	MM902	CM68	MM902	CM68	MM902	CM68	MM902
Grains					$2.07 \pm 0.09b$	2.47±0.09a	1.96±0.06b	$1.64 \pm 0.12c$
Glumes	$0.73 \pm 0.08b$	$0.55 \pm 0.04c$	$2.02 \pm 0.03a$	$0.80 \pm 0.08b$	$1.40 \pm 0.06a$	$1.07 \pm 0.12b$	$1.02 \pm 0.13b$	$0.53 \pm 0.03c$
Rachises	$0.36 \pm 0.07 b$	$0.22 \pm 0.01c$	$0.66 \pm 0.05a$	$0.35 \pm 0.04$ b	$0.24 \pm 0.02b$	$0.30 \pm 0.04$ a	$0.20 \pm 0.01$ c	$0.16 \pm 0.01d$
Internode I	$0.87 \pm 0.23$ b	$1.26 \pm 0.20a$	$1.49 \pm 0.20a$	$1.22 \pm 0.06a$	$0.59 \pm 0.02a$	$0.60 \pm 0.03a$	$0.42 \pm 0.04$ b	$0.31 \pm 0.03c$
Flag leaves	$0.68 \pm 0.03b$	$0.59 \pm 0.03c$	$1.17 \pm 0.02a$	$0.43 \pm 0.03$ d	$0.55 \pm 0.02a$	$0.34 \pm 0.01b$	$0.55 \pm 0.03a$	$0.23 \pm 0.01c$
Node I	$0.27 \pm 0.02c$	$0.13 \pm 0.02b$	$0.20 \pm 0.02b$	$1.08 \pm 0.22a$	$0.17 \pm 0.03a$	$0.11 \pm 0.01 \mathrm{b}$	$0.19 \pm 0.01a$	$0.08 \pm 0.01$ c
Lower inter- nodes	$1.78 \pm 0.09c$	$2.50 \pm 0.22b$	$3.51 \pm 0.35a$	$2.29 \pm 0.21$ b	$0.67 \pm 0.05b$	$0.43 \pm 0.03$ c	$0.91 \pm 0.02a$	$0.36 \pm 0.01$ d
Lower leaves	$2.16 \pm 0.12c$	$2.98 \pm 0.23b$	$3.65 \pm 0.11a$	3.61±0.09a	$1.57 \pm 0.13a$	$1.08 \pm 0.07$ b	$1.20 \pm 0.06b$	$0.54 \pm 0.01c$
Lower nodes	$0.42 \pm 0.03c$	$0.92 \pm 0.01$ b	$0.89 \pm 0.10b$	$1.66 \pm 0.15a$	$0.25 \pm 0.05$ ab	$0.21 \pm 0.01b$	0.29 ± 0.02a	$0.16 \pm 0.01c$
Roots	$1.12 \pm 0.17c$	$2.15\pm0.08\mathrm{b}$	$1.30 \pm 0.08c$	$2.48 \pm 0.05a$	$0.42 \pm 0.06a$	$0.24 \pm 0.02b$	$0.23 \pm 0.03b$	$0.19 \pm 0.02b$
Shoots	$7.27 \pm 0.17$ d	$9.14 \pm 0.30c$	13.59±0.51a	$11.44 \pm 0.37b$	$7.51 \pm 0.07a$	$6.61 \pm 0.32b$	$6.74 \pm 0.16b$	$4.01 \pm 0.06c$
Whole-plant	$8.39 \pm 0.30$ d	$11.29 \pm 0.23c$	$14.89 \pm 0.44a$	$13.92 \pm 0.41b$	$7.93 \pm 0.13a$	$6.85 \pm 0.34 \mathrm{b}$	$6.97 \pm 0.14 \mathrm{b}$	$4.20 \pm 0.05c$

lower leaves, and roots in CM68, and the Cd concentrations of glumes, rachises, node I, lower leaves, lower nodes, and roots in MM902 (Figs. S2A and 2B, E, G-I).

For tissue Cd content, at anthesis, the highest Cd content was found in lower leaves and lower internodes, followed by internode I and roots (Table 2). The Cd content of several upper tissues was significantly lower in MM902 than in CM68; the opposite results were found in several lower tissues regardless of urea-N or NPK-N application (Table 2). Compared with urea-N application, NPK-N application significantly increased the Cd contents of most tissues in both CM68 and MM902 (Table 2). At maturity, the highest Cd content was found in grains, followed by glumes and lower leaves (Table 2). The grain Cd content in MM902 was 2.47 µg/plant, which was higher than 2.07 µg/plant in CM68 under urea-N application; the opposite result was found under NPK-N application (Table 2). Additionally, the Cd contents of the glumes, flag leaves, node I, lower internodes, lower leaves and roots were higher in CM68 than in MM902 regardless of urea-N or NPK-N application (Table 2). Compared with urea-N application, NPK-N application did not change the Cd contents of grains, flag leaves, node I, or lower nodes in CM68, but significantly decreased those of other tissues in CM68 (except for lower internodes) by 10.25–45.24% and MM902 by 16.28–50.47% (Table 2).

Cd uptake and translocation of high- and low-Cd accumulators under urea-N and NPK-N applications

To reveal the physiological mechanisms by which NPK-N application limits grain Cd concentration, Cd uptake and root-to-shoot Cd translocation in MM902 and CM68 were analyzed. At anthesis and maturity, Cd uptake of MM902 was significantly lower than that of CM68 under urea-N and NPK-N applications (Fig. 4A, B). Compared with urea-N application, although NPK-N application significantly increased the Cd uptake of MM902 (23.95%) and CM68 (16.02%) at anthesis (Fig. 4A), it decreased that of MM902 and CM68 at maturity by 46.02% and 43.19%, respectively (Fig. 4B). The root-to-shoot Cd translocation factors of CM68 and MM902 were similar under urea-N and NPK-N application at anthesis and maturity(Fig. 4C, D), indicating that NPK-N

Fig. 4 Cd uptake and translocation at anthesis and maturity. A-B Whole-plant Cd uptake at anthesis and maturity of CM68 and MM902 under urea-N and NPK-N applications; C-D root-to-shoot Cd translocation at anthesis and maturity. Different letters indicate significance level at P < 0.05 by the Duncan's test



 Table 3
 Shoot Cd distribution and redistribution

Shoot	At anthesis				At maturity				During grain filling			
u listribu-	urea-N		NPK-N		urea-N		NPK-N		urea-N		NPK-N	
ion (%)	CM68	MM902	CM68	MM902	CM68	MM902	CM68	MM902	CM68	MM902	CM68	MM902
Grains					$26.21 \pm 0.51c$	37.41±0.89b	$33.76 \pm 1.45b$	42.41 ± 3.82a	$26.21 \pm 0.51c^{*}$	$37.41 \pm 0.89b^{*}$	$33.76 \pm 1.45 b^{*}$	42.41 ± 3.82a
Glumes	$9.98 \pm 0.82b$	$6.00 \pm 0.23c$	$14.86\pm0.45a$	$6.98\pm0.81c$	18.99±1.07a	$16.26 \pm 0.99b$	$14.18\pm1.33\mathrm{bc}$	$12.92 \pm 1.15c$	$9.00 \pm 1.48a^{*}$	10.26±0.89a*	$-0.69 \pm 1.74c$	$5.95 \pm 1.40b$
Rachises	$5.07 \pm 1.03a$	$2.36\pm0.10\mathrm{b}$	$4.89\pm0.41\mathrm{a}$	$3.07 \pm 0.33b$	$3.22 \pm 0.35c$	$4.51 \pm 0.18a$	$2.73 \pm 0.09 d$	$3.87 \pm 0.20b$	$-1.85 \pm 1.23c$	$2.15\pm0.13a^{*}$	$-2.16\pm0.37c^{*}$	$0.80 \pm 0.42b$
nter- node I	12.03±0.16ab	13.84±2.42a	10.96±1.02b	$10.72 \pm 0.86b$	7.98±0.27a	9.03±0.32a	$5.83 \pm 0.66b$	7.59 ± 1.06a	$-4.05\pm0.37a^{*}$	-4.81±2.68a*	$-5.13 \pm 1.52a^{*}$	$-3.13 \pm 0.70a$
rlag leaves	9.39±0.58a	$6.48 \pm 0.44b$	8.64±0.38a	$3.76 \pm 0.36c$	7.50±0.11a	$5.10 \pm 0.05b$	7.62±0.60a	$5.56 \pm 0.41 \mathrm{b}$	$-1.89\pm0.53b^{*}$	$-1.37\pm0.39b^{*}$	$-1.02 \pm 0.77b$	$1.80 \pm 0.27a$
Node I	$3.72 \pm 0.25 b$	$1.44 \pm 0.31c$	$1.43 \pm 0.10c$	$9.41 \pm 1.63 \mathrm{a}$	$2.28\pm0.38\mathrm{ab}$	$1.67\pm0.12bc$	2.58±0.04a	$1.90 \pm 0.19c$	$-1.43\pm0.15b^{*}$	$0.23 \pm 0.20$ ab	$1.14 \pm 0.06a^{*}$	$-7.50 \pm 1.80c$
Jower inter- nodes	24.43 ±0.70b	27.29 ± 1.49a	25.75±1.61ab	20.01±1.19c	9.08±0.64b	6.47±0.16c	12.60±0.47a	$8.76 \pm 0.65b$	$-15.35\pm0.28b^{*}$	$-20.81 \pm 1.60c^{*}$	−13.15±1.59ab*	$-11.25 \pm 1.56a$
leaves	29.59±1.21a	32.53 ± 1.59a	26.89±1.80b	31.55±0.89a	21.28 ± 1.38a	16.31±0.37b	$16.61 \pm 0.53b$	$13.17 \pm 0.51c$	$-8.30\pm0.89a^{*}$	$-16.22 \pm 1.54b^{*}$	$-10.27 \pm 2.33 a^{*}$	$-18.38 \pm 1.03b$
nodes	$5.81 \pm 0.50c$	$10.06 \pm 0.45b$	6.57±0.65c	14.51±1.43a	3.46±0.66a	3.24±0.09a	4.09±0.25a	3.83±0.17a	$-2.35 \pm 1.14a^{*}$	$-6.83 \pm 0.53 b^{*}$	$-2.48 \pm 0.72a^{*}$	$-10.69 \pm 1.57c$

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application did not alter the root-to-shoot Cd translocation of both CM68 and MM902.

Shoot Cd distribution factor of high- and low-Cd accumulators under urea-N and NPK-N applications

At anthesis, Cd was mainly distributed in lower leaves and internodes, followed by internode I and glumes in CM68 and MM902 (Table 3). The Cd distribution factors of several upper tissues (e.g., glumes, rachises, and flag leaves) were significantly lower in MM902 than in CM68, and the opposite results were observed in several lower tissues (e.g., lower leaves and lower nodes) under urea-N and NPK-N applications (Table 3). Compared with urea-N application, NPK-N application increased the Cd distribution factor of glumes and decreased those of node I and lower leaves in CM68; while, it increased the Cd distribution factors of node I and lower nodes, but decreased those of internode I, flag leaves, and lower internodes in MM902 (Table 3).

At maturity, Cd in shoots was mainly distributed in grains, followed by lower leaves and glumes (Table 3). The grain Cd distribution factors of CM68 were 26.21% and 33.76% under urea-N and NPK-N applications, respectively, which were significantly lower than those of MM902 of 37.41% and 42.41% under urea-N and NPK-N applications, respectively (Table 3). The opposite results were found for Cd distribution factors of glumes, flag leaves, node I, lower internodes, and lower leaves (Table 3). Compared with urea-N application, NPK-N application significantly increased the Cd distribution factors of grains in both CM68 (28.81%) and MM902 (13.37%), but decreased the Cd distribution factors of glumes, rachises, and lower leaves (Table 3).

## Shoot Cd redistribution of high- and low-Cd accumulators under urea-N and NPK-N applications

From anthesis to maturity, urea-N application increased the Cd distribution factors of grains and glumes in both CM68 and MM902 and rachises in MM902, but decreased those of other tissues in both cultivars (except node I of MM902) (Table 3). NPK-N application increased the Cd distribution factors of grains, glumes, rachises and flag leaves in MM902 and grains and node I in CM68, but decreased those

of other tissues in two cultivars (Table 3). However, the sum of the reduced Cd distribution factors of tissues below the spike (including lower nodes, lower leaves, lower internodes, node I, flag leaves and internodes) from anthesis to maturity was similar between urea-N and NPK-N applications for CM68 (33.37% with urea-N application and 30.91% with NPK-N application) and MM902 (49.81% with urea-N application). These results indicated that the Cd received in the spike from lower tissues redistribution was similar under urea-N and NPK-N applications, and that the differences were mainly in the competition of Cd redistribution in rachises, glumes and grains (Table 3).

Continuous Cd absorption/export of high- and low-Cd accumulators under urea-N and NPK-N applications

From anthesis to maturity, under urea-N application, the Cd content of per plant was significantly decreased from 8.39 to 7.93  $\mu$ g (a decrease of 0.48  $\mu$ g/plant) in CM68 and from 11.29 to 6.85  $\mu$ g/ plant (a decrease of 4.44  $\mu$ g/plant) in MM902 (Table 2). Under NPK-N application, the Cd content of per plant was significantly decreased from 14.89 to 6.97  $\mu$ g (a decrease of 7.92  $\mu$ g/plant) in CM68 and from 13.92 to 4.20  $\mu$ g (a decrease of 9.72  $\mu$ g/plant) in MM902 (Table 2). These results showed that during grain filling stage, MM902 had a higher continuous Cd export capacity than CM68; and NPK-N application promoted Cd export capacity in both MM902 and CM68 compared to urea-N application.

Relative expression levels of metal transporter genes of high- and low-Cd accumulators under urea-N and NPK-N applications

Our present physiological results showed that NPK-N application reduced grain Cd concentration via promoting Cd export to reduce Cd uptake, but not by altering root-to-shoot Cd translocation, shoot Cd distribution and redistrubition. Thus, the expression levels of genes responsible for Cd uptake (*TaIRT1, TaNRAMP5* and *TaZIP5*) and export (*TaCCX2* and *TaNRAMP5*) were further analyzed in the roots of CM68 and MM902 (Fig. 5). At anthesis, the expression level of *TaIRT1* was significantly lower in MM902 than in CM68, regardless of



Fig. 5 Relative expression of metal transporter genes. A-E Relative expression of *TaIRT1* (A), *TaZIP5* (B), *TaNRAMP5* (C), *TaCCX2* (D) and *TaNRAMP2* (E) in roots of CM68 and MM902 under urea-N and NPK-N application. F-I Relative

urea-N or NPK-N application (Fig. 5A). The expression levels of TaZIP5 and TaNRAMP5 were higher in MM902 than in CM68 under urea-N application, but there was no difference in that of TaNRAMP5 between CM68 and MM902 under NPK-N application (Fig. 5B, C). Importantly, NPK-N application only upregulated the expression of TaIRT1 in roots of MM902 and CM68 compared to urea-N application (Fig. 5A). The upregulation was consistent with the fact that NPK-N application promoted Cd uptake at anthesis in both cultivars (Table 2; Fig. 4A). Thus, TaIRT1 might be responsible for the Cd uptake at anthesis, which was regulated by NPK-N application. At grain filling stage, the expression of TaCCX2 in the roots of MM902 was significantly higher than that of CM68, regardless of N application; and NPK-N application also upregulated the expression of TaCCX2 in both cultivars compared to urea-N application (Fig. 5D). Thus, TaCCX2 may be related to the continuous Cd export from the plant during grain filling. Besides, the expression of TaN-RAMP2 was similar between CM68 and MM902 under both urea-N and NPK-N application, indicating that it was not responsible for the differences in Cd efflux between the two cultivars (Fig. 5E).

To explain the different Cd redistribution of spikes, we also analyzed the expressions of several

expression of *TaNRAMP3* (**F**), *TaHMA2* (**G**) and *TaLCT1* (**H**) in node I, rachises and glumes of CM68 and MM902 under urea-N and NPK-N application. Different letters indicate significance level at P < 0.05 by the Duncan's test

metal transporter genes involved in Cd distribution and export in node I, rachises and glumes of CM68 and MM902 at the grain filling stage. The expression levels of *TaNRAMP3* were higher than those of *TaHMA2* and *TaLCT1* in both cultivars under urea-N and NPK-N applications (Fig. 5F-H). NPK-N application did not affect *TaNRAMP3* expression in node I, but downregulated that in rachises and glumes of both CM68 and MM902 compared to urea-N application (Fig. 5F), which was consistent with the decrease of Cd redistribution in rachises and glumes. However, the expression levels of *TaHMA2* and *TaLCT1* regulated by NPK-N application were not consistent with Cd redistribution in these tissues (Fig. 5G, H).

### Overexpression of TaIRT1 in rice

Since the function of wheat *CCX2* in Cd efflux was demonstrated in our recent study (Cheng et al. 2024), three *TaIRT1*-overexpressing rice lines were constructed in this study to test whether it transports Cd in cereals (Fig. S4A). Compared with the wide-type Zhonghua 11, overexpression of *TaIRT1* did not alter plant height, tiller number, branches number, grain length, grain width, thousand grain weight, grain number per plant, and grain yield per plant (Fig. S4B-I). However, overexpression of



**Fig. 6** Tissue Cd concentration, Cd uptake, root-to-shoot translocation, and shoot-to-grain Cd distribution in *TaIRT1* overexpressing lines. **A** Tissue Cd concentration; **B** whole-plant Cd uptake; **C** root-to-shoot Cd translocation; **D** shoot-

to-grain Cd distribution. "\*" and "ns" indicate significant difference and no significant difference between WT and *TaIRT1* overexpressing lines, respectively

*TaIRT1* significantly increased Cd concentrations in grains, lower leaves, lower nodes and roots and Cd uptake, whereas it decreased root-to-shoot Cd translocation, shoot-to-grain Cd distribution, and Cd concentrations in glumes, rachises, internode I, flag leaves and node I (Fig. 6A-D).

### Discussion

Grain Cd concentration in wheat cultivars controlled by genetic factors and influenced by environmental factors

Here, three-year field experiments were performed to evaluate the effects of NPK-N application on grain Cd concentration using 50 wheat cultivars (Fig. 1). Among the 50 cultivars, only two cultivars, MM902 and Aikang 58, were defined as low-Cd accumulators, which could be used as important germplasm resources for low-Cd genetic improvement. Nineteen cultivars, such as CM68, accumulated high levels of Cd in grains at all experiments, exhibiting environmental-stable and high-Cd accumulation. Another 29 cultivars showed unstable grain Cd concentration in different years. These results indicated that the grain Cd concentration of wheat showed genotypic variation; and that the grain Cd concentration of different wheat genotypes was not only controlled by genetic factors but also influenced by environmental factors. These results were similar to previous evaluations for Japanese wheat cultivars and American winter wheat cultivars (Kubo et al. 2008; Guttieri et al. 2015).

Low Cd uptake before anthesis and high continuous Cd export during grain filling contribute to low grain Cd concentration in MM902

Grain Cd concentration in cereal crops is determined by Cd uptake before anthesis, root-to-shoot Cd translocation, shoot Cd distribution, and continuous Cd absorption/export and redistribution to grains via the phloem during grain filling (Tavarez et al. 2015; Kubo et al. 2016; Yan et al. 2018, 2019; Cheng et al. 2021). In this study, the grain Cd concentration of MM902 was lower than that of CM68, regardless of urea-N or NPK-N application (Fig. 3A). Further analysis revealed that the low grain Cd concentration of MM902 was due to lower Cd uptake before anthesis and higher Cd export from plants during grain filling (Fig. 4A; Table 2). Thus, at maturity, Cd uptake was lower in MM902 than in CM68 (Fig. 4B). The difference in Cd uptake between CM68 and MM902 was not related to inter-root soil characteristics or root dry weight, as these were similar between the two cultivars (Table 1; Table S1). The lower Cd uptake before anthesis in MM902 may result from the lower expression level of *TaIRT1* than that of CM68 (Fig. 5A). The homologous gene of TaIRT1, OsIRT1, is responsible for Cd uptake in rice (Nakanishi et al. 2006; Yang et al. 2016), and TpIRT1 in Polish wheat localized in the plasma membrane acts as a Cd influx transporter in yeast (Jiang et al. 2021). Importantly, overexpression of TaIRT1 in rice significantly increased wholeplant Cd uptake and grain Cd concentration (Fig. 6A, B). In addition, the higher Cd export in MM902 during grain filling may result from the higher expression level of TaCCX2 than that of CM68 at the grain filling stage (Fig. 5D). Its homologous gene, OsCCX2, is involved in Cd export in rice (Hao et al. 2018); and overexpression of wheat CCX2 reduced whole-plant Cd uptake and grain Cd concentration in rice (Cheng et al. 2024). Since continuous Cd absorption during grain filling contributed approximately half of the Cd concentration in durum wheat grains (Harris and Taylor 2013; Yan et al. 2018, 2019), Cd export from the plant during grain filling should be one of the important reasons for limiting grain Cd concentration.

The difference in grain Cd concentration did not result from root-to-shoot Cd translocation, owing to

it being similar between CM68 and MM902 (Fig. 4C, D). This also did not result from the Cd distribution to grains during grain filling, owing to the grain Cd distribution factor of MM902 at maturity was significantly higher than that of CM68 (Table 3). The higher grain Cd distribution in MM902 could be due to the lower Cd distribution in glumes, flag leaves, node I, lower internodes, and lower leaves (Table 3), which are potential sources of Cd redistribution to grains during grain filling (Kubo et al. 2016; Cheng et al. 2021). The results indicated that the effect of low Cd uptake before anthesis and high Cd export during grain filling may be greater than the effect of high Cd distribution to grains, resulting in lower grain Cd concentration in MM902.

# NPK-N application reduces grain Cd concentration in CM68 and MM902 by promoting Cd export during grain filling

Our previous study showed that NH<sub>4</sub><sup>+</sup>-N application did not change Cd uptake or grain Cd concentration in high-Cd accumulating wheat cultivar, but increased grain Cd concentration in low-Cd accumulating wheat cultivar by promoting continuous Cd absorption and transport to grains during grain filling (Cheng et al. 2021). Other studies have found that urea-N application generally causes increased grain Cd concentrations in winter and durum wheat, and NO<sub>3</sub><sup>-</sup>-N application affects grain Cd concentrations differently in different wheat species (Wangstrand et al. 2007; Ata-UI-Karim et al. 2020; Perilli et al. 2010; Tosun et al. 2023). However, why these phenomena occur is poorly understood. Meanwhile, these results also indicate that urea-N,  $NH_4^{+-}N$ , and NO<sub>3</sub><sup>-</sup>-N are not suitable cultivation management practices for reducing grain Cd concentrations in wheat. In this study, NPK-N application significantly reduced grain Cd concentrations in all 50 wheat cultivars collected from different wheat regions with different genetic backgrounds; and most of the wheat cultivars under NPK-N application accumulated lower Cd concentrations than the international standards (0.20 mg/kg) when grown on Cd-contaminated soils (Fig. 1). Thus, NPK-N application has an advantage in reducing grain Cd concentration in wheat with different genetic backgrounds.

Although NPK-N application promoted the preanthesis Cd uptake of CM68 and MM902 when compared with urea-N application (Fig. 4A), it significantly promoted continuous Cd export during grain filling (Table 2) and finally resulted in a sharp decrease in Cd uptake at maturity (Fig. 4B). These results indicated that Cd export during grain filling promoted by NPK-N application was the main physiological process for limiting grain Cd concentration. This export could be caused by the up-expression of TaCCX2 in roots of CM68 and MM902 under NPK-N application when compared with urea-N application (Fig. 5D). Meanwhile, NPK-N application slightly increased pH and decreased available Cd concentration in the soil compared with urea-N application (Table S1), which theoretically supports that NPK-N application inhibits Cd uptake (Kashiwagi et al. 2009; Cheng et al. 2017; Yan et al. 2023). However, the preanthesis Cd uptake in CM68 and MM902 were higher with urea-N application than with NPK-N application (Fig. 4A). Thus, the effect of soil characteristics on Cd uptake in wheat was smaller than the uptake capacity of wheat, which may resulted from the upregulation of the Cd absorber gene TaIRT1 (Fig. 5A).

NPK-N application did not affect root-to-shoot Cd translocation but increased shoot-to-grain Cd distribution of CM68 and MM902 when compared with urea-N application (Fig. 4C, D; Table 3). The increase in grain Cd distribution was not related to grain Cd concentration, but to changes in Cd distribution from shoots to spikes during grain filling. In wheat, Cd redistribution from lower tissues is usually transported to spikes via xylem or phloem, and rachises and glumes of spikes are phloem sinks that compete with grains during grain filling (Kubo et al. 2016; Yan et al. 2018). In this study, the sum of the reduced Cd distribution factors of tissues below the spike from anthesis to maturity was similar between urea-N and NPK-N applications for CM68 and MM902 (Table 3). However, the Cd distribution factors of glumes and rachises at maturity were lower under NPK-N application than under urea-N application in both cultivars (Table 3). Thus, the lower Cd distribution factors of rachises and glumes in MM902 resulted in a higher Cd distribution in the grains under NPK-N application. Metal transporters are the main factors involved in the xylem-to-phloem Cd transfer and tissue Cd export (Yang et al. 2020). NPK-N application downregulated the expression of TaNRAMP3 in rachises and glumes of CM68 and MM902 (Fig. 5F). Wheat NRAMP3, encoding a plasma membrane-localized metal transporter for Cd influx (Peng et al. 2018a), is mainly expressed in nodes and leaves responsible for Cd distribution to rachises and glumes (unpublished data).

Furthermore, NPK-N application increased the grain (106.61%) and whole-plant (77.43%) dry weights of CM68 but did not change those of MM902 compared with urea-N application (Table 1). Thus, the dilution effect of grain biomass on the reduction in the grain Cd concentration in CM68 could not be excluded; however, it was not significant (Ismael et al. 2018; Saha et al. 2022).

### NPK-N application improves biomass accumulation and yield

Nitrogen is the principal limiting factor for crop yield (Li et al. 2022a). The effects of different forms of N on wheat yield were dependent on the wheat cultivar. For most wheat cultivars, urea-N application can improve wheat yield more than NO<sub>3</sub><sup>-</sup>-N or NH<sub>4</sub><sup>+</sup>-N application (Lyu et al. 2022; Cui et al. 2023). Therefore, the application of appropriate forms of N fertilizer can be an effective method to optimize grain yield (Cui et al. 2023). In this study, NPK-N application significantly increased grain yield and whole-plant dry weight of CM68 compared with those of urea-N application (Fig. 2L; Table 1). Thus, CM68 is preferred to NPK-N application in improving yield and biomass accumulation. Although NPK-N application also improved grain yield and whole-plant biomass of MM902 compared with urea-N application, the improvement was not significant (Fig. 2L; Table 1). Thus, MM902 differs from CM68 in N fertilizer preference, and MM902 has a higher potential for yield improvement. Our study also showed that grain yield of 50 wheat cultivars were higher under NPK-N application than under urea-N application (Fig. S2). These results suggest that NPK-N application is an effective strategy for biomass accumulation and yield improvement independent of wheat cultivar.

In wheat, yield improvement by improving the source-sink partitioning of N is often associated with a high grain number per plant but not with grain weight (Philipp et al. 2018; Meng et al. 2021; Lyu et al. 2022). In this study, NPK-N application



The effects of urea-N and NPK-N application on Cd transport in wheat

Fig. 7 The potential transport pathway of urea-N and NPK-N application on Cd transport in wheat

did not alter the spikelet number per spike, grain length and width, or thousand grain weight of CM68 when compared with the urea-N application (Fig. 2G-J). Thus, the increase in grain yield in CM68 caused by NPK-N application resulted from an increase in grain number (Fig. 2K). This result is consistent with the finding that NPK-N application increased the grain-setting rate of wheat (Ferrante et al. 2013).

### Conclusion

Taken together, our results showed that NPK-N application can limit grain Cd concentration and increase grain yield of wheat. It mainly promotes Cd export from plant to soil during grain filling stage, thus inhibiting Cd uptake at maturity. Meanwhile, the different grain Cd concentrations between CM68 and MM902 are mainly results from the different Cd uptake before anthesis and Cd export during grain filling stage. The potential transport pathway of NPK-N reduced grain Cd concentration was observed in Fig. 7. These results provide theoretical guidance and technical support for high-yield and high-quality wheat production. We also defined two low-Cd accumulators, MM902 and Aikang 58, which are important resources for the improvement of low-Cd accumulated cultivars.

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#### Declarations

**Competing interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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