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Characteristics of cadmium uptake and membrane transport in roots of intact wheat (*Triticum aestivum* L.) seedlings[☆]

Lian-Zhen Li^a, Chen Tu^a, Willie J.G.M. Peijnenburg^{b,c}, Yong-Ming Luo^{a,*}

^a Key Laboratory of Coastal Zone Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences (CAS), Shandong Provincial Key Laboratory of Coastal Zone Environmental Processes, YICCAS, Yantai, PR China

^b National Institute of Public Health and the Environment, Center for Safety of Substances and Products, P.O. Box 1, 3720 BA Bilthoven, The Netherlands

^c Institute of Environmental Sciences (CML), Leiden University, Leiden, The Netherlands

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ABSTRACT

Wheat is one of several cereals that is capable of accumulating higher amounts of Cd in plant tissues. It is important to understand the Cd²⁺ transport processes in roots that result in excess Cd accumulation. Traditional destructive technologies have limited capabilities in analyzing root samples due to methodological limitations, and sometimes may result in false conclusions. The mechanisms of Cd²⁺ uptake into the roots of wheat seedlings (*Triticum aestivum* L.) were investigated by assessing the impact of various inhibitors and channel blockers on Cd accumulation as well as the real-time net Cd²⁺ flux at roots with the non-destructive scanning ion-selective electrode technique. The P-type ATPase inhibitor Na₃VO₄ (500 μM) had little effect on Cd uptake ($p < 0.05$) and the kinetics of transport in the root of wheat, suggesting that Cd²⁺ uptake into wheat root cells is not directly dependent on H⁺ gradients. While, the uncoupler 2,4-dinitrophenol significantly limited Cd²⁺ uptake ($p < 0.05$) and transport kinetics in the root of wheat, suggesting the existence of metabolic mediation in the Cd²⁺ uptake process by wheat. The Cd content at the whole-plant level in wheat was significantly ($p < 0.05$) decreased upon pretreatment with the Ca²⁺ channel blockers La³⁺ or Gd³⁺ and Verapamil, but not in case of pretreatment with the K⁺ channel blocker tetraethylammonium (TEA). In addition, the inhibitors of the Ca²⁺ channel, as well as high concentrations of Ca²⁺, reduced the real-time net Cd²⁺ fluxes at the root surface in SET experiments. These results indicate that Cd²⁺ moves across the plasma lemma of the wheat root via Ca²⁺ channels. In addition, our results suggested a role for protein synthesis in mediating Cd²⁺ uptake and transport by wheat.

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1. Introduction

Cadmium (Cd²⁺) is a highly toxic trace element that is present in all soils. Due to high Cd mobility and bioavailability in the soil-plant system it can easily enter the food chain, representing a potential health risk. As compared with other cereals, wheat (*Triticum aestivum* L.) tends to accumulate more Cd in plant tissues when grown in soils that contain elevated levels of this toxic metal (Hart et al., 1998; Puschenreiter et al., 2005). A maximum permissible concentration of Cd in wheat grain has been set by national and international health authorities in response to the potential health

risks of Cd accumulation (FAO/WHO, 2001; European Commission, 2001; GB 2762, 2012; Australia New Zealand Standard 1.4.1, 2013).

To allow for control of Cd uptake in this crop species it is important to understand the physiological and biological processes that result in excess Cd accumulation. To identify possible pathways for Cd²⁺ uptake, the uptake and transport of Cd²⁺ at the root surface has been extensively characterized in wheat (Hart et al., 1998). Due to the relatively poor spatial and temporal resolution of traditional techniques, most reports have failed to identify the underlying uptake mechanisms involved (Newman, 2001). Measurement of specific ion fluxes using ion-selective microelectrodes has contributed significantly to the identification and functional characterization of the transporter (Newman et al., 1987; Shabala et al., 1997; Smith et al., 1994; Kochian et al., 1992; Pineros et al., 1998). The ion-selective microelectrode technique to measure specific ion fluxes non-invasively is ideally suited for this purpose,

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* Corresponding author. No. 17, Chunhui Road, Yantai 264003, PR China.

E-mail addresses: lianzen.li@yic.ac.cn (L.-Z. Li), ctu@yic.ac.cn (C. Tu), Willie.Peijnenburg@rivm.nl (W.J.G.M. Peijnenburg), ymlo@yic.ac.cn (Y.-M. Luo).

given its key features of e.g. being non-invasive and displaying high spatial and temporal resolution (Newman, 2001).

As a non-essential element for plants, Cd has been assumed to be taken up by transporters for essential elements as a consequence of lack of specificity of the transporters (Pence et al., 2000; Cohen et al., 1998; Connolly et al., 2002). Potassium and calcium are the very important inorganic macronutrients of the living cell. Numerous reports have focused on the alleviation of Cd phytotoxicity and accumulation by Ca and K (Suzuki, 2005; Ismail, 2008; Zorrig et al., 2012). Nevertheless, the underlying mechanism of effects of essential elements on Cd²⁺ toxicity and accumulation is still unclear, although some hypothetical mechanisms to interpret the interactions of Ca²⁺ or K⁺ with Cd²⁺ (Kinraide, 1998; Yang and Juang, 2015; Liu et al., 2014) have been proposed. There is little direct evidence in support of the possibility of uptake of Cd²⁺ by channels that are permeable to Ca²⁺ or K⁺ in wheat root. Thus, the present study was designed to determine the specificity of the Cd transport system by examining the effect of pharmaceuticals as well as of Ca or K on Cd uptake and real-time Cd²⁺ fluxes at the roots of intact wheat seedlings with the application of non-destructive scanning ion-selective electrode technique.

For this purpose, we measured the Cd content in whole wheat seedlings with pre-treatment of a range of pharmaceuticals (including a metabolic inhibitor and ion channel blockers) as well as in the presence of cations. To verify the uptake and transport mechanisms thus identified, we also monitored the real-time net Cd²⁺ fluxes at the root surface using the scanning ion-selective electrode technique (SIET). By assessing the effects of the metabolic inhibitor dinitrophenol (DNP) and the H⁺-ATPase inhibitor Na₃VO₄ on Cd accumulation or real-time net Cd²⁺ fluxes at the root, we investigated if Cd²⁺ uptake and transport were dependent on metabolic energy or on H⁺-gradients. To test whether Ca²⁺ and K⁺ channels are involved in the membrane transport system of wheat, or whether other transport mechanisms occur, we measured the Cd content in the plants. Thereupon, we determined the real-time net Cd²⁺ fluxes at the roots in the presence and absence of Ca²⁺ and K⁺, as well as with treatment of Ca²⁺ (La³⁺, Gd³⁺ or Verapamil) and K⁺ (tetraethylammonium, TEA) channels blockers. In addition, the potential role of phytochelatin (PC) and protein synthesis on Cd uptake by wheat (*Triticum aestivum* L.) was evaluated by blocking PCs and protein production via L-Buthionine-sulfoximine (BSO) and cycloheximide (CHX), respectively.

2. Materials and methods

2.1. Plant material culture

To investigate possible Cd²⁺ uptake pathways into wheat root, bread wheat (*Triticum aestivum* L.) variety Jimai 22, which has been widely planted in northern China, was used in this study. Plants of wheat were grown as described elsewhere (Hart et al., 1998). In brief, seeds were surface sterilized and germinated on sterile filter paper. Germinated seedlings were transferred to black polyethylene cups positioned in light-sealed black 5 L polyethylene pots containing continuously aerated Hoagland solution (pH at 6.0 ± 0.2) that was replaced once every two days. Seedlings were grown for 8 days in a climate-controlled room at 25 °C and 18 °C during 16/8 h light/dark, and healthy plant seedlings of uniform size were selected for the subsequent experiments.

2.2. Cd accumulation upon treatment of metabolic inhibitors and PC and protein synthesis inhibitors

The inhibitors were mixed with the Hoagland solution to achieve their following final concentrations: DNP, 50 μM; Na₃VO₄,

500 μM; BSO, 250 μM; CHX, 20 μM. These concentrations were based on results of previous literature reports demonstrating they are physiologically relevant (Pang et al., 2006; Zhang et al., 2012; Zeng et al., 2009).

The seedlings were pre-treated with solutions containing the pharmacological agents (pH 6.0) for 12 h before the uptake experiment. Pre-treatment in pharmacological free solution was used as the control treatment. After pre-exposure, the seedlings were rinsed twice and then were transferred to Hoagland solutions containing 10 μM Cd in 1 L HDPE containers. One seedling was transplanted into each container. There were four containers for each treatment as replicates. Plants were harvested after 7 d, washed in 1.0 mM EDTA, rinsed with deionized water, oven-dried, weighed and digested with HNO₃. Cd concentrations in the digest were determined by ICP-MS (Agilent 7500i, Agilent Technologies Co. Ltd, USA). GBW07605 tea leaves (State Bureau of Technical Supervision, People's Republic of China) were used as certified reference materials for quality control of Cd determination. Measured concentrations agreed well with the certified concentrations for Cd.

2.3. Effect of ions and ion channel blockers on uptake of Cd

In order to investigate the effect of Ca and K channel blockers on Cd uptake by *Triticum aestivum* L., an uptake experiment was conducted similarly as described above. The seedlings were pre-exposed in solutions containing 1 mM LaCl₃, 100 μM GdCl₃, 50 μM Verapamil or 100 μM TEA for 12 h prior to the uptake experiment. The experimental procedure used was the same as described above.

To further test if Ca and K channels or other ion channels were involved in Cd²⁺ transport in the plasma membrane, an experiment was conducted to assess the effect of exogenous ions including Ca, Mg, Na and K on Cd uptake by *Triticum aestivum* L. Intact seedlings of *Triticum aestivum* L. were treated with 10 μM Cd, and with different concentrations of Ca²⁺, Mg²⁺, Na⁺ and K⁺ respectively in Hoagland nutrient solution. CaCl₂ or MgSO₄ was added to the background solution to provide three test solutions of 0.1, 1.0, and 10 mM of Ca or Mg (Ca or Mg-set), while NaCl or KCl was added to the background solution to provide three test solutions of 2.5, 5.0, and 10.0 mM Na or K (Na or K-set).

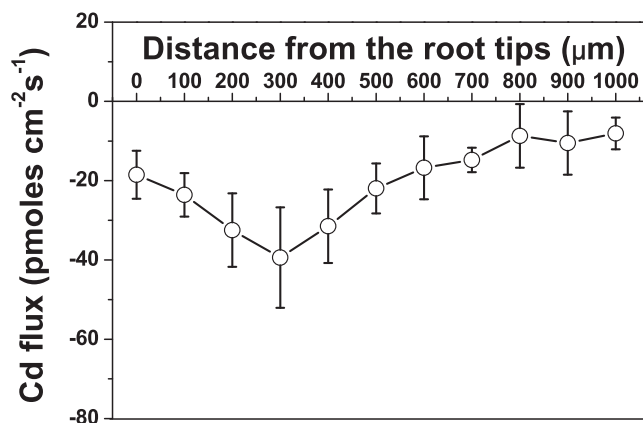


Fig. 1. Illustration of the magnitude of Cd²⁺ fluxes (mean ± standard error) around root tips of wheat *Triticum aestivum* L. Negative values indicate influx from solution to the root. Flux measurements were carried out in 10 μM Cd(NO₃)₂ at different positions along the root apex. Each point represents the mean of six seedlings and bars represent the standard error of the mean, measured at each position. Roots were scanned in segments of 100 μm from the root tip.

2.4. Net Cd^{2+} fluxes measurement at the root surface of *Triticum aestivum* L.

Net fluxes of Cd^{2+} at the surface of wheat roots were measured non-invasively with the application of scanning ion selective electrode technique (BIO-001A; Younger USA, LLC, MA, USA), essentially as described by Shabala et al. (1997, 2001). Details on fabrication and calibration of Cd^{2+} ion selective microelectrodes have been described previously (Ma et al., 2010).

To monitor net fluxes of Cd^{2+} at different positions along the root apex of *Triticum aestivum* L., an initial scanning measurement was carried out at the root tip followed by 100 μm walk steps. With the plant intact, the primary (longest) root of each plant was selected and mounted horizontally in the measuring chamber, and loosely fixed in place with dental wax. The chamber was then filled with measuring solution consisting of 0.1 mM $\text{Cd}(\text{NO}_3)_2$, 0.1 mM KCl, 0.1 mM CaCl_2 , 1.0 mM NaCl, 0.1 mM MgSO_4 , and 0.15 mM 2-(N-

Morpholino) ethanesulfonic acid (MES) with pH of 6.0 and equilibrated for 10 min. The equilibrated root was then used to record the net Cd^{2+} flux at each position in fresh measuring solution.

2.5. Transient net Cd^{2+} fluxes at the root surface of *Triticum aestivum* L. with treatment of a metabolic inhibitor and PC and protein synthesis inhibitor

The transient Cd flux at the root position where the most intense Cd flux usually occurs was recorded before and after the treatment with the pharmaceuticals. The primary roots of seedlings were equilibrated in the measuring solution for 10 min and then a steady-state Cd flux was recorded using SET for 10 min before the addition of pharmaceutical. A stock solution of the pharmaceutical selected was slowly added to the measuring solution to yield a final concentration that was the same as in the accumulation experiment describe above. Afterward, the measuring solution was

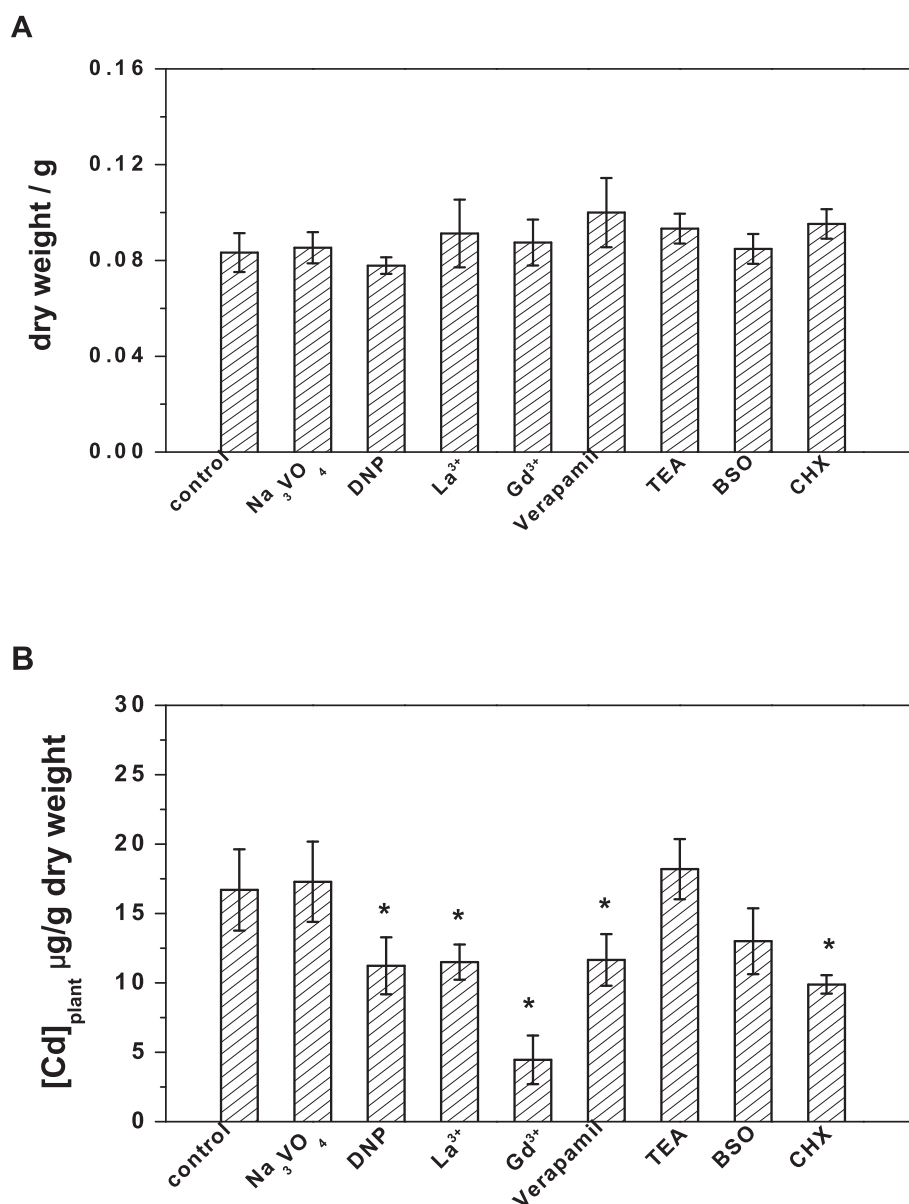


Fig. 2. Effect of various inhibitors on plant dry weight (A) and Cd accumulation (B) in whole seedlings of wheat *Triticum aestivum* L. after 7 d exposure in Hoagland solutions containing 10 μM $\text{Cd}(\text{NO}_3)_2$. Plants seedlings were pre-exposed to pharmaceuticals for 12 h, controls consist of pharmaceuticals-free solutions. Values are means \pm S.D. ($n = 4$). * Indicates significant differences in metal accumulation from controls ($p < 0.05$).

refreshed and the Cd flux recording was continued at the same position along the root as described above. For each treatment, at least six net Cd^{2+} flux measurements were made.

2.6. Transient net Cd^{2+} fluxes at the root surface of *Triticum aestivum* L. with treatment of ion channel blockers as well as in the presence of different level of inorganic cations

We determined the fluxes of Cd^{2+} at the root surface of wheat by treatment with a series of ion channel blockers: La^{3+} , Gd^{3+} and Verapamil as well as TEA were used to modify the activity of Ca^{2+} and K^{+} channels, respectively. The transient Cd flux at the root position where the most intense Cd flux usually occurs was recorded using SIET before and after the treatment with the pharmaceuticals as described above.

To further verify the possible effects of different ions on Cd uptake by *Triticum aestivum* L., the real-time net Cd^{2+} fluxes were measured using SIET in measuring solution with 0.1, 1.0, and 10 mM Ca^{2+} or Mg^{2+} , as well as 2.5, 5.0, and 10.0 mM Na^{+} or K^{+} at the position where intense Cd flux usually occurs.

2.7. Statistical analysis

Data were analyzed by one-way Analysis of Variance (SPSS version 13.0 software for Windows). Data were shown as mean \pm standard error (S.E.M.). Duncan's Multiple Range test at the 5% level of probability was used to compare the differences in metal concentrations between treatments.

3. Results

As can be seen from Fig. 1, the Cd^{2+} flux profile showed a clear spatial organization and it was indicated that the highest Cd^{2+} influx was localized at positions 300 μm from the root apex, with a steadily decreasing influx at all positions above and below this site.

To test whether the H^{+} -ATPase plays a role in energizing Cd uptake, we used the P-type ATPase (including H^{+} -ATPase) inhibitor Na_3VO_4 to inhibit H^{+} -ATPase in the plant roots. The concentration of Cd in the whole plant was not affected by pre-treatment with 500 μM Na_3VO_4 in solution ($P < 0.05$) (Fig. 2). The real time Cd^{2+} transport kinetics results showed that Na_3VO_4 did not inhibit the Cd uptake rate at the roots surface (Fig. 3). Treatment with 10 μM 2,4-dinitrophenol (DNP), a metabolic inhibitor, resulted in considerable reduction of the Cd influx at the root at the same position of 300 μm from the root apex (Fig. 3). Meanwhile, pre-treatment of wheat roots by DNP, caused the Cd contents of wheat to decrease by 33% (Fig. 2, $p < 0.05$).

To further understand the pathways of Cd uptake and membrane transport in roots of wheat, we determined the Cd contents in wheat seedlings upon pretreatment with ion channel blockers and found that the Ca^{2+} channels blockers La^{3+} or Gd^{3+} as well as verapamil, at the whole-plant level, significantly decreased Cd levels ($p < 0.05$) (Fig. 2). Unlike the Ca^{2+} channel blocker, pre-treatment with the K^{+} channel blocker TEA had little effect on Cd uptake in plants ($p < 0.05$) (Fig. 2). In addition, the real-time net Cd^{2+} flux into root was reduced in SIET experiments with the treatments of La^{3+} or Gd^{3+} and Verapamil, but not in case of TEA (Fig. 4). These findings indicate that the uptake of Cd into the root of *Triticum aestivum* L. proceeds through channels permeable to Ca^{2+} .

To further verify the Cd^{2+} membrane transport mechanism across the root of *Triticum aestivum* L., we investigated the effect of inorganic cations on the uptake and real-time kinetics of the Cd flux in the roots. The presence of Ca was found to significantly inhibit Cd uptake ($p < 0.05$, Fig. 5), reducing Cd concentration in the plants from 18.8 $\mu\text{g g}^{-1}$ dry weight at 0.1 mM Ca to 12.3 $\mu\text{g g}^{-1}$ and

8.4 $\mu\text{g g}^{-1}$ dry weight at 1.0 and 10.0 mM Ca, respectively (Fig. 5A). Similar to Ca, increased Mg concentrations resulted in lower Cd contents (Fig. 5A). Our results also showed that Cd uptake by *Triticum aestivum* L. was not reduced by increasing K^{+} and Na^{+} concentrations (Fig. 6A).

Addition of 1.0 mM and 10.0 mM Ca^{2+} to the measuring solution significantly decreased the net Cd^{2+} influx in roots, reducing by 16% and 72% at 300 μm back from the root apex, respectively (Fig. 5B, $p < 0.01$). However, when the Mg^{2+} concentration in the measuring solution was increased to 10.0 mM, the net Cd^{2+} flux into the root was changed from a net influx to a net efflux (Fig. 5B). The addition of Na^{+} or K^{+} in to the measuring solution had on the other hand no effect on the net Cd^{2+} flux at the root surface, even when the concentration of either Na^{+} or K^{+} in the measuring solution was increased to 10 mM (Fig. 6B).

To further investigate whether the uptake and transport of Cd into the root of *Triticum aestivum* L. are related to PCs synthesis and protein synthesis, L-Buthionine-sulfoximine (BSO), a blocker of PC synthesis, and cycloheximide (CHX), a kind of protein synthesis inhibitor, were used in the present study. Pre-treatment with BSO had no effects on the Cd content in the whole plant ($p < 0.05$, Fig. 2) and the transient net Cd^{2+} fluxes at the roots surface were not changed significantly upon treatment with BSO (Fig. 7B). The mean of the net influx of Cd^{2+} was 47.0 $\text{pmol cm}^{-2} \text{ s}^{-1}$ and the CHX treatment decreased the net influx of Cd^{2+} to 20.8 $\text{pmol cm}^{-2} \text{ s}^{-1}$ at 300 μm from the root apex (Fig. 7A). In addition, pre-treatment of CHX significantly inhibited Cd uptake in the whole plant ($p < 0.05$, Fig. 2).

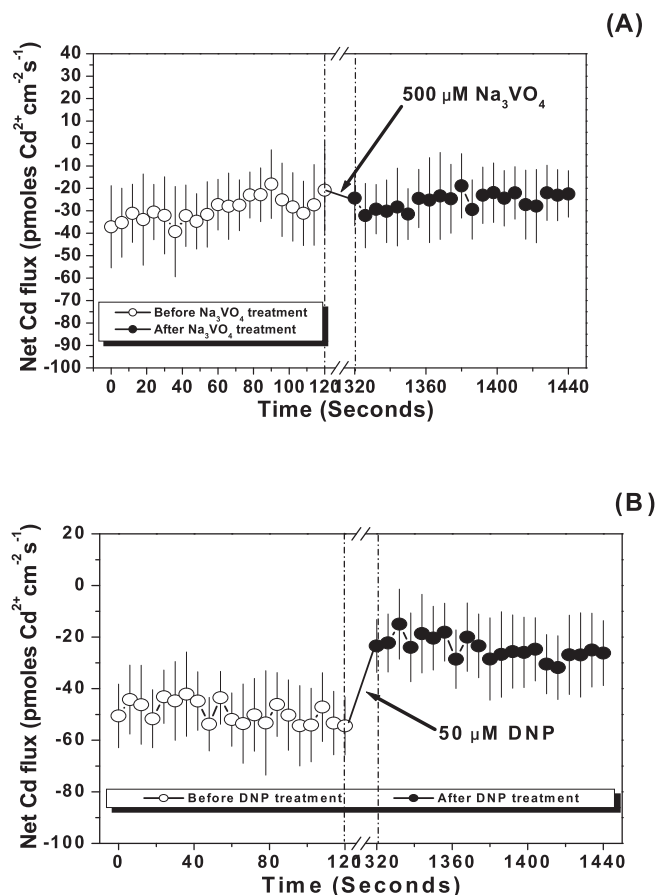


Fig. 3. Transient net Cd^{2+} flux at the root position 300 μm from the root apex (where intense Cd flux usually occurs) of wheat *Triticum aestivum* L. before and after applications of 500 μM Na_3VO_4 (A) and 50 μM dinitrophenol (DNP) (B).

4. Discussion

4.1. Localization of Cd^{2+} fluxes along the root apex of wheat

The observed decrease of the net flux of Cd^{2+} at the root surface with increasing distance from the root tip is consistent with the research of Pineros et al. (1998). Pineros et al. (1998) found that the Cd^{2+} flux into the root of bread wheat was greatest in the region of about 0.6–1.2 mm from the root apex and decreased with increasing distance from the root apex. However, in our study, the most vigorous Cd^{2+} flux was observed in the apex region of 0–0.7 mm with a highest value at around 0.3 mm from the root tip (Fig. 1). These data indicate that the spatial patterns of the net Cd^{2+} flux along roots in these two studies are probably different between the wheat types or varieties. Farrell et al. (2005) have reported that there were differences in the net Cd^{2+} flux at the root surface of different wheat cultivars. This spatial difference in net Cd^{2+} flux may be attributed to differences in root anatomy (Dong et al., 1995) or localization of the Cd^{2+} uptake system (Page and Feller, 2005) between these wheat types or varieties. Although a similar Cd^{2+} flux pattern was also observed in the present study, the net Cd^{2+} flux was greater than that observed by Farrell et al. (2005) for durum wheat using the same Cd^{2+} selective microelectrode. Using ^{109}Cd radiotracer flux techniques, Hart et al. (1998) compared

uptake of Cd^{2+} in seedlings of bread and durum wheat cultivars and they found that the bread wheat cultivar had higher root Cd^{2+} uptake rates. Nevertheless, available evidences indicate that the grains Cd concentrations in durum wheat were higher as compared to that in bread wheat (Zook et al., 1970; Meyer et al., 1982). These results suggested that the high Cd-accumulation in grain of durum wheat could not be solely attributed to high Cd influxes at the root surface.

4.2. The effect of metabolic inhibitors on Cd uptake and real-time kinetics transport at the root of *Triticum aestivum* L.

All membranes P-type ATPase could be inhibited by Na_3VO_4 . Pre-treatment of wheat roots by Na_3VO_4 did not significantly inhibit Cd uptake in our study (Fig. 2), suggesting that Cd uptake into the root of wheat was not dependent upon physiological functions of the plasma membrane P-type ATPase.

As an uncoupler of oxidative phosphorylation, DNP has the ability to break down proton gradients by increasing proton permeability of biomembranes leading to inhibition of ATP biosynthesis (Felle and Bentrup, 1977; Tripathi et al., 1995). The significant suppression of the Cd influx by the metabolic inhibitor DNP suggests the existence of metabolic mediation in the Cd^{2+} uptake process by wheat. Cd entry into the root involves a

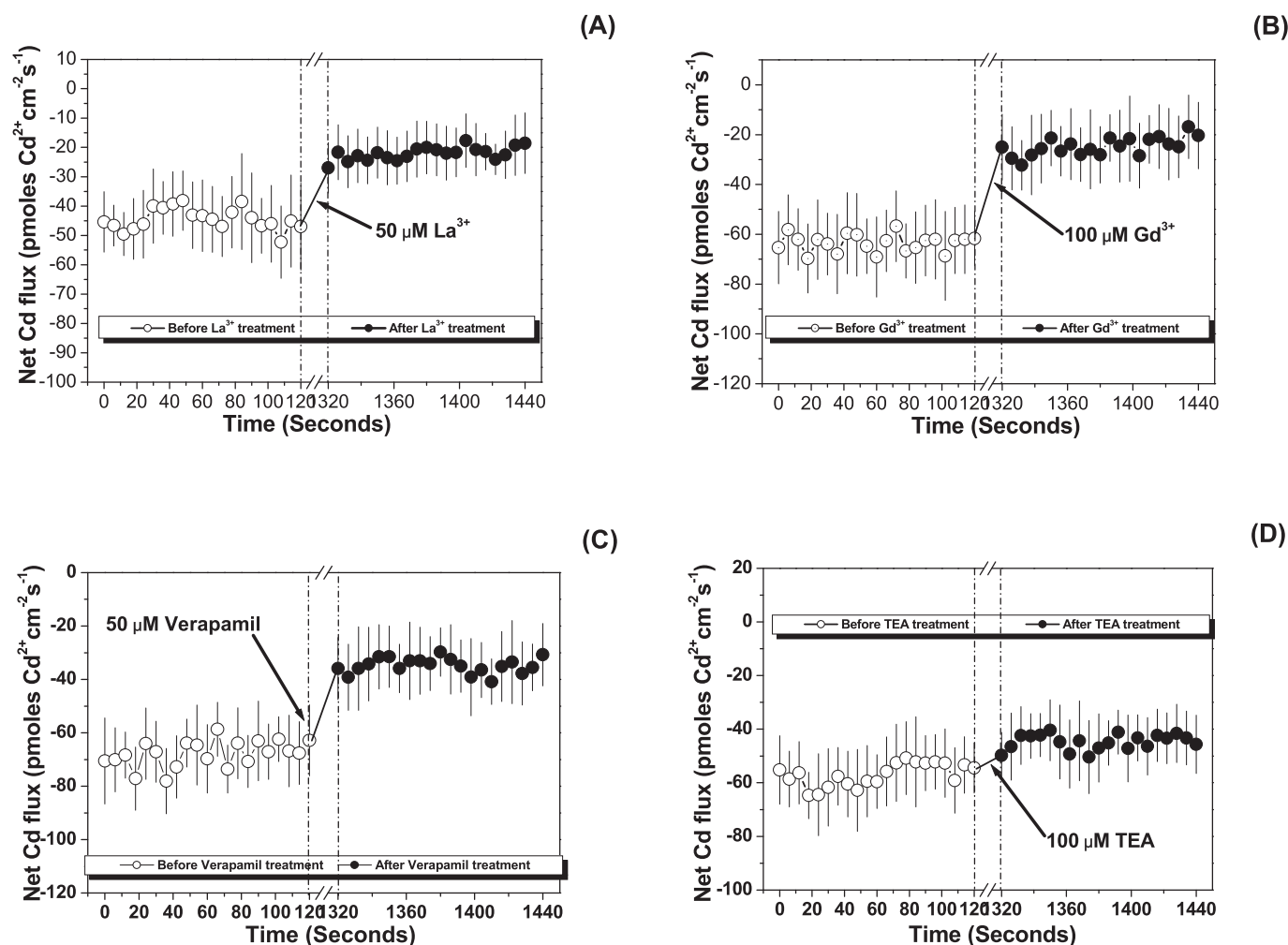


Fig. 4. Transient net Cd^{2+} flux at the root position 300 μm from the root apex (where intense Cd flux usually occurs) of wheat *Triticum aestivum* L. before and after applications of 50 μM La^{3+} (A), 100 μM Gd^{3+} (B), 50 μM Verapamil (C) and 100 μM tetraethylammonium (TEA) (D).

symplastic pathway that is dependent on metabolic energy. Many studies have shown that inhibition of metabolism affects the uptake of Cd, in which the symplastic pathway rather than the apoplastic pathway is believed to play an important role. The observation of inhibition of Cd uptake by the metabolic inhibitor DNP led to the conclusion that the metabolism plays an important role in the movement of Cd²⁺ into the root cells of soybean (*Glycine max*) (Cataldo et al., 1983). Similarly, most of the Cd translocated from roots to the shoot in 'high' isolines of durum wheat probably via the symplastic pathway (Van der Vliet et al., 2007; Quinn et al., 2011).

4.3. The effect of ion channel blockers on Cd uptake and real-time kinetics transport at the root of *Triticum aestivum* L.

To test the potential effect of Ca²⁺ permeable channels on Cd uptake, the Cd content was measured in wheat seedlings pre-treated with Ca²⁺ channel blockers. Our experiments with inorganic channel blockers showed that Cd accumulation was strongly inhibited by La³⁺ and Gd³⁺, as well as by the organic Ca²⁺ channel blocker verapamil (Fig. 2). The results of Clemens et al. (1998) also

showed that the Cd²⁺ uptake activity of the transporter LTC1 in wheat was blocked by La³⁺ and Ca²⁺. Nevertheless, the K⁺ channel blocker TEA did not suppress the Cd uptake (Fig. 2, $p < 0.05$). In addition, the Cd content in plant seedlings decreased with increasing Ca²⁺ and Mg²⁺ concentrations, but not in the presence of different concentrations of Na⁺ and K⁺ (Figs. 6 and 7). Thus, Cd²⁺ is likely taken up by wheat through channels permeable to Ca²⁺, as confirmed by the results showing reduced real-time net Cd²⁺ fluxes at the root surface upon treatment with Ca²⁺ channel blockers, as well as in the presence of high concentrations of Ca²⁺ (Fig. 6). In other report, it was found that Cd could enter guard cell protoplasts via Ca²⁺ channels, but not by K⁺ channels (Perfus-Barbeoch et al., 2002). The results of Lindberg et al. (2004) also showed that Cd uptake into the cytosol of wheat protoplasts partly takes place by channels permeable to Ca²⁺. These results supported our finding that the uptake of Cd²⁺ by wheat (*Triticum aestivum* L.) is mediated at least in part by Ca²⁺ channels. By measuring the real-time kinetics of Cd transport using SIET, we herein provided direct evidence of the regulation of Cd²⁺ uptake by Ca²⁺ transporters in root cell plasma membranes of bread wheat.

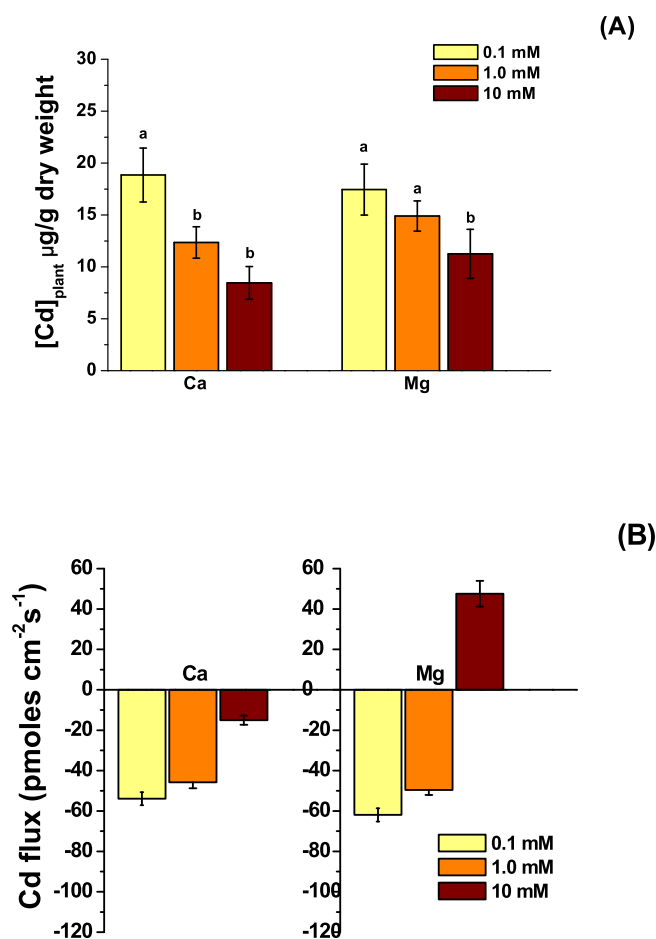


Fig. 5. Cd uptake and real-time net Cd fluxes on the root of wheat *Triticum aestivum* L. in the presence of different concentration of Ca or Mg. (A) In the uptake experiment, the Hoagland solution containing 10 µM Cd was used as the background nutrient solution. Values are means \pm S.D. ($n = 4$). Bars with different letters are significantly different ($p < 0.05$). (B) Mean rates of Cd fluxes during record in the different concentrations of Ca or Mg. Columns labeled with different letters are significantly different ($p < 0.05$). The net Cd²⁺ fluxes were measured at the position 300 µm (where intense Cd flux usually occurs) back from the root apex.

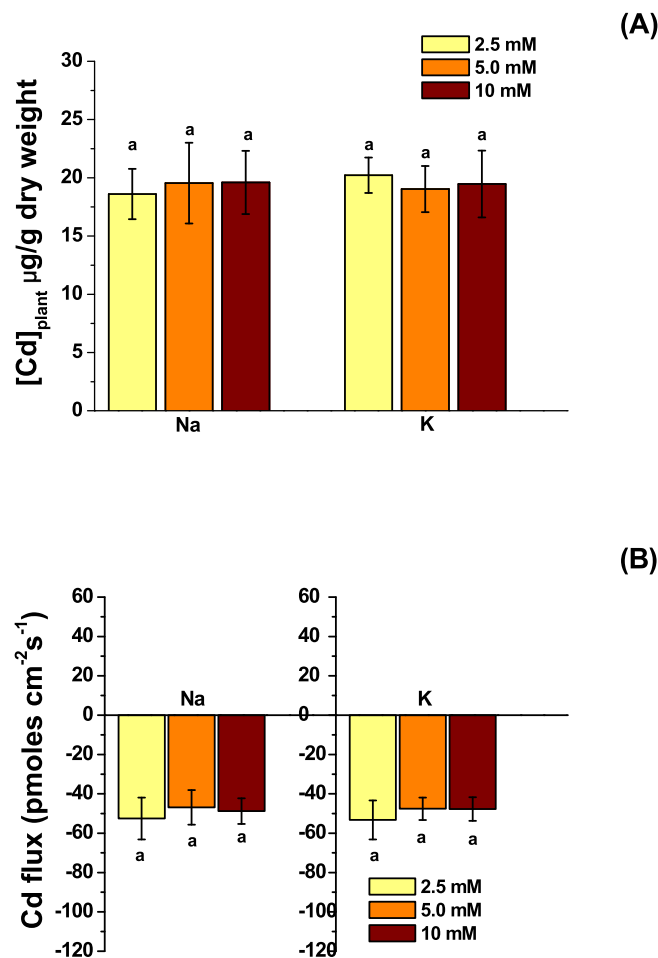


Fig. 6. Cd uptake and real-time net Cd fluxes on the root of wheat *Triticum aestivum* L. in the presence of different concentration of Na or K. (A) In the uptake experiment, the Hoagland solution containing 10 µM Cd was used as the background nutrient solution. Values are means \pm S.D. ($n = 4$). Bars with different letters are significantly different ($p < 0.05$). (B) Each column represents the mean of four individual plants. The bars represent the standard error of the mean. Columns labeled with different letters are significantly different ($p < 0.05$). The net Cd²⁺ fluxes were measured at the position 300 µm (where intense Cd flux usually occurs) back from the root apex.

4.4. Effect of phytochelatin (PC) and protein synthesis inhibitors on Cd uptake and real-time kinetics transport at the root of *Triticum aestivum* L.

The role of PCs in Cd tolerance has been well characterized in various plants and other living organisms (Zenk, 1996; Cobbett, 2000), but the role of PCs in uptake of Cd is still unclear. In the present study, the potential role of PCs synthesis on Cd uptake by *Triticum aestivum* L. was evaluated by blocking PCs production via L-Buthionine-sulfoximine (BSO). Pre-treatment of BSO had no effects on the Cd content in the plants ($p < 0.05$) (Fig. 2), which is confirmed by the fact that treatment with BSO did not reduce the real-time net Cd fluxes at the root surface as deduced using SIET (Fig. 7A). The results obtained suggested that PC synthesis bears no relationship to the uptake of Cd^{2+} into wheat roots. On the other hand, the results from Lindberg et al. (2004) indicated that pre-treatment with Cd^{2+} (with increased PCs content) decreased the short-term uptake of Cd^{2+} into the cytosol of wheat protoplasts. In other report, it was also found that accumulation of Cd after 24 h increased in yeast cells transformed with a PCS1 gene

(phytochelatin synthase 1 gene) from wheat (Clemens et al., 1998).

To further investigate whether the transport of Cd into the root of wheat takes place via specific transport proteins, the root was treated with a typical protein synthesis inhibitor CHX (Seo et al., 2009). Upon CHX treatment, the Cd^{2+} uptake and influxes in the roots decreased significantly, indicating that specific transport proteins are involved in Cd^{2+} uptake by wheat. Thus, except for the ion channels (White, 1996), ion pumping and carriers might be involved in the passage of ions through the plasma membranes of wheat. Further biochemical and molecular studies are required to identify and document the role of ion channels and specific transport proteins in Cd^{2+} uptake and transport on the root of *Triticum aestivum* L. All the concentrations of the pharmaceuticals used in this study were selected based on previous literature reports showing that these concentrations are physiologically relevant (Tripathi et al., 1995; White, 1996; Zhang et al., 2012). In our experiment the seedlings showed no obvious plant toxicity symptoms and the mean weight of individual plants was comparable among the different treatments (Fig. 2A). It is reasonable to propose that the differences in Cd absorption among different treatments

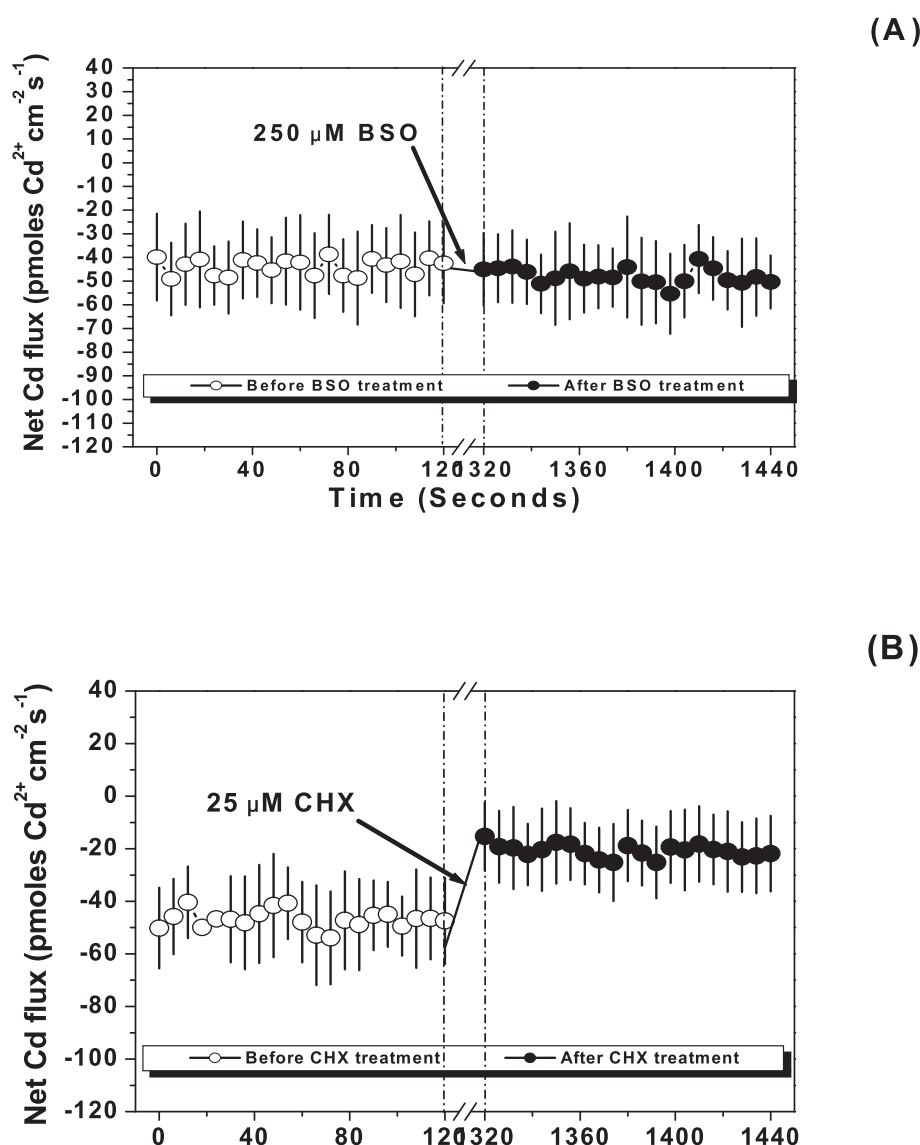


Fig. 7. Transient net Cd^{2+} flux at the root position 300 μm from the root apex (where intense Cd flux usually occurs) of wheat *Triticum aestivum* L. before and after applications of 250 μM l-Buthionine-sulfoximine (BSO) (A) and 25 μM cycloheximide (CHX) (B).

may be attributed to the direct effect of various pharmaceuticals.

In summary, uptake and the real-time kinetics transport of Cd^{2+} in roots of *Triticum aestivum* L. was significantly suppressed by metabolic inhibitors, which demonstrated that uptake of Cd^{2+} by roots of *Triticum aestivum* L. depends on metabolic energy. Ca^{2+} channel blockers as well as high concentrations of Ca^{2+} decreased Cd uptake and the real-time net Cd^{2+} fluxes at the root surface, indicating that uptake of Cd^{2+} by *Triticum aestivum* L. is related to Ca^{2+} channels. In addition, our results suggest a role of protein synthesis in mediating Cd^{2+} uptake by *Triticum aestivum* L. Inhibition of Cd^{2+} uptake by selected pharmaceuticals and inorganic cations was confirmed by direct evidence of assessment of real time Cd^{2+} fluxes at the root surface, as performed using SIET. These findings are helpful for us to understand the Cd^{2+} uptake pathways and the physiological processes that result in excess Cd accumulation in wheat.

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