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ORIGINAL ARTICLE

Cadmium translocation and accumulation in developing barley grains

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Abstract Soil cadmium (Cd) contamination has posed a serious problem for safe food production and become a potential agricultural and environmental hazard worldwide. In order to study the transport of Cd into the developing grains, detached ears of two-rowed barley *cv*. ZAU 3 were cultured in Cd stressed nutrient solution containing the markers for phloem (rubidium) and xylem (strontium) transport. Cd concentration in each part of detached spikes increased with external Cd levels, and Cd concentration in various organs over the three Cd levels of 0.5, 2, 8 μM Cd on 15-day Cd exposure was in the order: awn > stem > grain > rachis > glume, while the majority of Cd was accumulated in grains with the proportion of 51.0% relative to the total Cd amount in the five parts of detached spikes. Cd accumulation in grains increased not only with external Cd levels but the time of exposure contrast to stem, awn, rachis and glume. Those four parts of detached spike showed increase Cd accumulation for 5 days, followed by sharp decrease till day 10 and increase again after 12.5 days. Awn-removal and stem-girdling markedly decreased Cd concentration in grains, and sucrose or zinc (Zn) addition to the medium and higher relative humidity (RH) also induced dramatic reduction in Cd transport to developing grains. The results indicated that awn, rachis and glume may involve in Cd transport into developing grains, and suggested that Cd redistribution in maturing cereals be considered as an important physiological process influencing the quality of harvested grains. Our results suggested that increasing RH to 90% and Zn addition in the medium at grain filling stage would be beneficial to decrease Cd accumulation in grains.

Keywords Barley (*Hordeum vulgare* L) · Cadmium · Humidity · Phloem/xylem transport · Sucrose · Zinc

Abbreviations

Cd	Cadmium
Zn	Zinc
Rb	Rubidium
Sr	Strontium
RH	Relative humidity

Introduction

Cadmium (Cd) is potentially toxic to both plants and animals and has no beneficial biological function in the aquatic or terrestrial organism. Recently, Cd accumulation in biotic systems as a consequence of human activities is becoming a major environmental issue worldwide; particularly in agricultural ecosystems, where it might endanger crop productivity and quality (Sandalio et al. 2001; Wu et al. 2003, 2004; Lima et al. 2006; Chen et al. 2007). Cd is suggested to cause damage even at very low concentrations (Järup et al. 1998), and healthy plants may contain levels of Cd that are toxic to mammals (Herren and Feller 1997). For safe food production it would be beneficial and cost-effective to develop crop cultivars with high resistance/tolerance to

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Cd toxicity and simultaneously with low Cd accumulation in the edible parts. It is thus imperative to elucidate the mechanism of Cd accumulation in edible parts of plants for developing low Cd accumulation cultivars to minimize soil-to-plant transfer of Cd.

Cadmium accumulation in plants is regulated by several physiological processes, including Cd uptake from soil (via roots) and atmosphere (via shoot surface), xylem translocation from root to shoot and phloem movement into grain during maturation (Hart et al. 1998). The difference of grain Cd contents in bread and durum wheat cultivars was suggested to be based on the genotypic variations in root uptake (Stolt et al. 2003) and the root-to-shoot transport of Cd (Oliver et al. 1995; Penner et al. 1995; Clarke et al. 1997). In their study of durum wheat using near isogenic lines, Buckley et al. (1997) found that low grain Cd levels were closely related to more Cd retention in roots. However, it has been shown that higher Cd accumulation in durum wheat relative to bread wheat was not the direct result from increased uptake of Cd by roots or its xylem translocation from roots to shoots (Hart et al. 1998). Higher Cd accumulation in durum wheat grains is possibly related to the higher capacity of durum wheat to translocate more Cd via the phloem into grains (Herren and Feller 1997). These authors found that Cd partially reached the ear (and especially the grains) via phloem, and the relative mobility in the phloem and the xylem-to-phloem transfer in peduncle or in lower internodes are important for Cd accumulation in maturing wheat grains. Studies of durum wheat resulted in valuable information on the phloem mobility of Cd (Cakmak et al. 2000a, b; Harris and Taylor 2001; Tanaka et al. 2003). Harris and Taylor (2004) suggested that restricted root-to-shoot Cd translocation may limit Cd accumulation in durum wheat grain by either directly controlling Cd translocation from roots during grain filling, or by the size of shoot Cd pools that can be remobilized to the grain. Cd is probably either translocated directly via the xylem to the grains during maturity or translocated with the bulk stream of photosynthates from leaves to grains via the phloem (Greger and Löfstedt 2004). The discrepancies about the relative importance of root uptake and root-to-shoot translocation in grain Cd accumulation may attribute to the difference of genotypes used in the experiments, and partially reflect differences in experimental condition. More detailed information is therefore required about the physiological processes responsible for Cd redistribution/accumulation in grains (Harris and Taylor 2004).

External factors, such as transpiration [e.g. relative humidity (RH)], and micronutrient cations may also affect Cd absorption and distribution between different plant parts. Zinc (Zn) is chemically very similar to Cd and significant interactions can occur between Cd and Zn in their accumulation by plants (Chesworth 1991). Yet the available reports provided conflicting results. For example, Choudhary et al. (1995) and Oliver et al. (1994) reported that an increase of Zn level in growth media reduced the Cd accumulation in grains at least under some circumstances, e.g. when wheat was grown on Zn-deficient soil. Similarly, Zn reduced both Cd uptake by roots and xylem transport of Cd from roots to shoots in lettuce and spinach (McKenna et al. 1993). Hart et al. (2002) revealed a competitive interaction between Cd and Zn root uptake in bread and durum wheat seedlings, suggesting that Cd and Zn may share a common transport system at the root cell plasma membrane. However, other studies showed either no interaction (White and Chaney 1980) or even evidences of synergism (Root et al. 1975; Cataldo et al. 1983; Jalil et al. 1994) between Cd and Zn. In addition, the loading of Zn ions was markedly affected by sucrose mass flow in the phloem in seedlings of *Phaseolus vulgaris* (Rauser and Samarakoon 1980). Maintaining wheat spikelets at high RH reduced transpiration and xylem transport, and almost completely blocked Zn transport into grains (Pearson et al. 1996). Therefore the question arises whether the transport of Cd is similar to the transport of Zn, and whether Zn, sucrose mass flow and environmental humidity may interfere with Cd transport to developing grains.

Apart from the results on wheat mentioned above, only limited information is available on Cd accumulation and retranslocation in barley grains, despite the fact that this plant is one of the major cereal crops in the world. Thus, a precise knowledge about the physiological basis of Cd accumulation in barley grains would be useful. The present study reported the dynamic accumulation, remobilization and redistribution of Cd in developing barley spikes. Moreover, we studied the effect of Zn nutrition, sucrose mass flow, awn removal and the change of environmental humidity to elucidate the mechanism of Cd accumulation in grains and how it might be affected by some environmental factors. On the other hand, strontium (Sr) and rubidium (Rb) were reported as the markers of the xylem and phloem transport, respectively (Feller 1989; Zeller and Feller 1999), and we used these two elements to distinguish between xylem and phloem transport.

Materials and methods

Plant materials

Two rowed barley (*Hordeum vulgare cv*. ZAU 3, kindly provided by the breeder, Professor Ding Shouren, Department of Agronomy, Zhejiang University, China) was grown in the experimental farm on Huajiachi Campus (Zhejiang University, Hangzhou, China). Spikes with uniform size and similar growth stage were chosen and tagged

at anthesis. The tagged spikes were cut from the stem 15–20-day post-anthesis ~15 cm below the flag leaf node followed immediately by further cutting under water with a razor-blade until only 10 cm below the node remained (Jenner 1985). Then spikes with flag leaves were removed while sheaths were intact, were transferred to sterilized circular glass jars containing 150 ml nutrient solution, and sealed by aluminium foil and covered with polystyrol-plates with seven evenly spaced holes. In each hole one spike was inserted, placed in a growth incubator with light intensity of 200 μm m^{-2} s^{-1} at ear level and day/night temperature of 22 ± 0.5°C/18 ± 0.5°C with 15 h of day time. The composition of the basic nutrient solution was (mg l^{-1}): $(NH_4)_2SO_4$, 48.2; $MgSO_4$, 65.9; K_2SO_4, 15.9; KNO_3, 18.5; $Ca(NO_3)_2$, 59.9; KH_2PO_4, 24.8; Fe-citrate, 5; $MnCl_2{\cdot}4H_2O$, 0.9; $ZnSO_4{\cdot}7H_2O$, 0.11; $CuSO_4{\cdot}5H_2O$, 0.04; HBO_3, 2.9; and H_2MoO_4, 0.01. The solution pH was adjusted to 6.5 ± 0.1 with HCl or NaOH, as required.

Experimental design

Spikes were treated as follows: (1) Dynamic of Cd accumulation and distribution—$CdCl_2$ was added to the basic solution to form 0 (control), 0.5, 2 or 8 μM Cd levels under 60% RH. The nutrient solution was renewed in 5-day interval and the spikes were washed in water and cut about 1 cm at the bottom of the shoots before retransferred into circular flasks, and the spikes were collected after 1, 5, 10, 12.5 and 15-day Cd exposure. (2) The effect of humidity—spikes were cultured at three levels of 30, 60 and 90% RH; Cd as $CdCl_2$ was added to each container to form four concentrations of 0 (control), 0.5, 2 and 8 μM. (3) The effect of awn removal/stem girdling—awn is an important structure to increase the surface area for transpiration. In this treatment, the awns were removed from the lemma of ears with sharp scissors to determinate its role in xylem transport. For stem-girdling the stems were heat girdled by hot water ~1 cm below the spikes for 20 s. The treatment killed all living cells in a 1–1.5 cm long section of the stem and interrupted the phloem, while the xylem remained functional. The samples and the control without awn and stem treatment were cultured in nutrition solutions containing 2 or 8 μM Cd, together with 5 μM $SrCl_2$ and 5 μM RbCl as markers for xylem and phloem transport (Feller 1989; Zeller and Feller 1999), under 60% RH. (4) The effect of sucrose addition—spikes were collected 24 h prior to Cd addition and kept in basic culture solution in dark, and then cultured in the nutrition solution supplemented with 2 μM Cd, 5 μM $SrCl_2$ and 5 μM RbCl and 0 (control), 30 or 150 mM sucrose under 60% RH. (5) The effect of Zn addition—spikes were cultured in nutrient solution supplemented with 2 μM Cd, 5 μM $SrCl_2$ and 5 μM RbCl and 0 (absence in Zn, i.e. basic solution without Zn addition), 100 or 300 μM $ZnSO_4$ under 60% RH. The spikes of the 2–5 experiments mentioned above were collected on the fifth day after treatment. There were three replicates in each of the last four cases and 15 replicates for the first case, and each replicate contained seven spikes.

Measurements and statistical analysis

Each shoot was dried at 80°C and divided into the following parts: grain, awn, stem, glume (including palea, lemma and glume), rachis (including rachillas and sterile florets). The plant parts were powdered and weighted, then ashed at 550°C for 12 h. The ash was digested with 5 ml 30% HNO_3, and diluted using deionized water. Cd, Zn, Sr and Rb concentrations were determined using a flame atomic absorption spectrometry (Shimadzu AA-6300; Fang 1991). The data was subjected to ANOVA, and then Duncan's multiple-range test (SSR) was used for testing the mean difference between treatments by using the Data Processing System (DPS) statistical software package (Tang and Feng 1997).

Results

Dynamics of Cd uptake and distribution

The dose- and time-responses for Cd concentrations in different parts of barley shoots are summarized in Fig. 1. Cd concentration in each part of detached shoots increased with external Cd level. We detected the most dramatic effect in the presence of the highest solution Cd concentration but the same tendencies were observed with the lower levels as well. The Cd concentration of glume, awn, rachis and stem (Fig. 1a–d) increased sharply in the first 5 days with the peaks at 5 days, after that decreased till 12.5 days and increased again after 12.5 days but only the Cd concentration of the awn reached the level measured at day 5. However, grain Cd concentration pattern was different (Fig. 1e). The Cd concentration of grains increased sharply in the first 5 days, became nearly steady with a slight increase during 5–15-day Cd exposure, and the maximum Cd concentration was recorded in 15-day Cd exposure. Cd concentration in various plant parts over the three Cd levels at 15-day Cd exposure was in the order: awn > stem > grain > rachis > glume. The uptake rate, however, mainly depended on the solution level of Cd, and tissue Cd concentration increased with external Cd level. For instance, at 15-day Cd exposure, Cd concentration in stem, awn, grain, glume and rachis was 3.9, 12.0, 9.3, 8.6, 4.9-fold higher in 8 μM Cd treatment than 0.5 μM Cd treatment, respectively.

As shown in Fig. 2, similar trend was observed in Cd accumulation based on per ear as Cd concentration, i.e. Cd

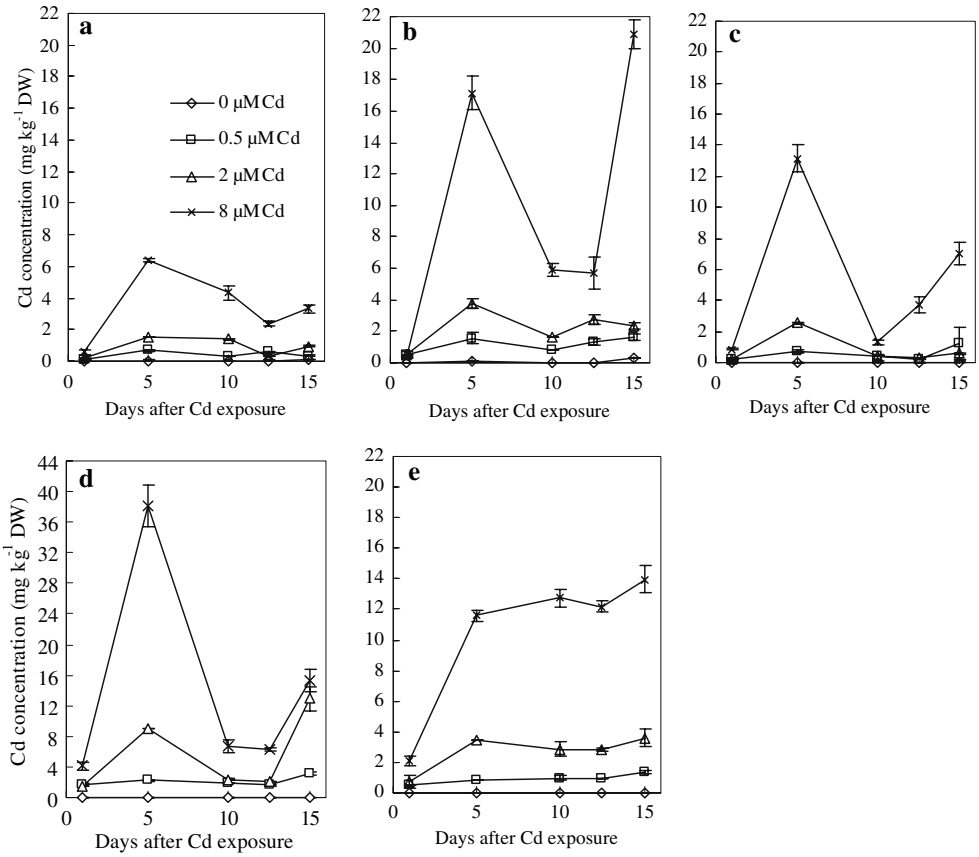

Fig. 1 Kinetics of Cd concentration of different part of barley shoots after 1, 5, 10, 12.5 and 15 days cultured under different Cd levels. Glume (**a**), awn (**b**), rachis (**c**) stem (**d**) and grain (**e**). Mean values of three independent replicates and each replicate containing seven shoots (mean ± SD, $n = 3$)

accumulation in glumes, awns, rachides and stems of detached shoots (Fig. 2a–d) increased sharply over the first 1–5-day Cd exposure when all parts, except awn, had the highest Cd accumulation, while decreased during 10–12.5 days (with the exception of rachis Cd content in day 12.5 being higher than that in day 10 but still markedly lower than that in day 5), and after that Cd accumulation tended to increase again. However, grain Cd accumulation increased with both external Cd level and exposition time (Fig. 2e). There was a marked difference in the proportion of Cd accumulation in the different organs, relative to the total Cd amount in the tissues, with the grains showing the largest proportion (48.9, 51.8, 52.3% in 0.5, 2, 8 μM Cd treatments after 15-day exposure, respectively), followed by awn/stem (correspondingly 19.3, 10.4, 26.0%, and 17.0, 29.5, 9.6%), and rachis/glume being the smallest (correspondingly 10.2, 1.9, 6.2%, and 4.7, 6.4, 6.0%). On the other hand, Cd accumulation increased with increasing Cd level in the solution, and the trend remained throughout the duration of Cd exposure. Thus, on average of the five sampling dates Cd contents in grains, awns, stems, glumes and rachides increased by 1.9, 0.8, 1.7, 1.8, 0.7-folds for the shoots exposed to 2 μM Cd treatment, and by 9.0, 6.6, 7.3, 8.4, 9.8-folds in 8 μM Cd treatment, respectively, compared with that of 0.5 μM Cd treatment.

Effect of relative humidity on Cd uptake and distribution

The influence of RH on Cd distribution in spikes is shown in Fig. 3. High RH (90%) induced significant ($P < 0.05$) reduction in Cd concentration of grain, awn and glume of the spikes exposed to 0.5, 2 and 8 μM Cd (by 39.5, 53.8, 41.0%, and 50.6, 61.0, 61.1% on average of the three Cd treatments), and of rachis in 2, and 8 μM Cd (by 54.7 and 44.5% on average of the two Cd treatments, respectively), compared with that in 30, and 60% (RH) treatments, while an increase was observed in rachis Cd concentration of 0.5 μM Cd treatment with 90% RH over the 30% RH ($P > 0.05$) and 60% RH ($P < 0.05$) treatments, and no significant difference in controls among the three RH. On the other hand, average over the three Cd levels Cd concentrations in both of grains and glumes of spikes under 30% RH were 18.0 and 33.5% lower than that in 60% RH, but 66.0, and 69.9% higher than that in 90% RH. Hence, more Cd would be accumulated in the grains and glumes in RH 60% treatment compared to the other two RH treatments (Fig. 3).

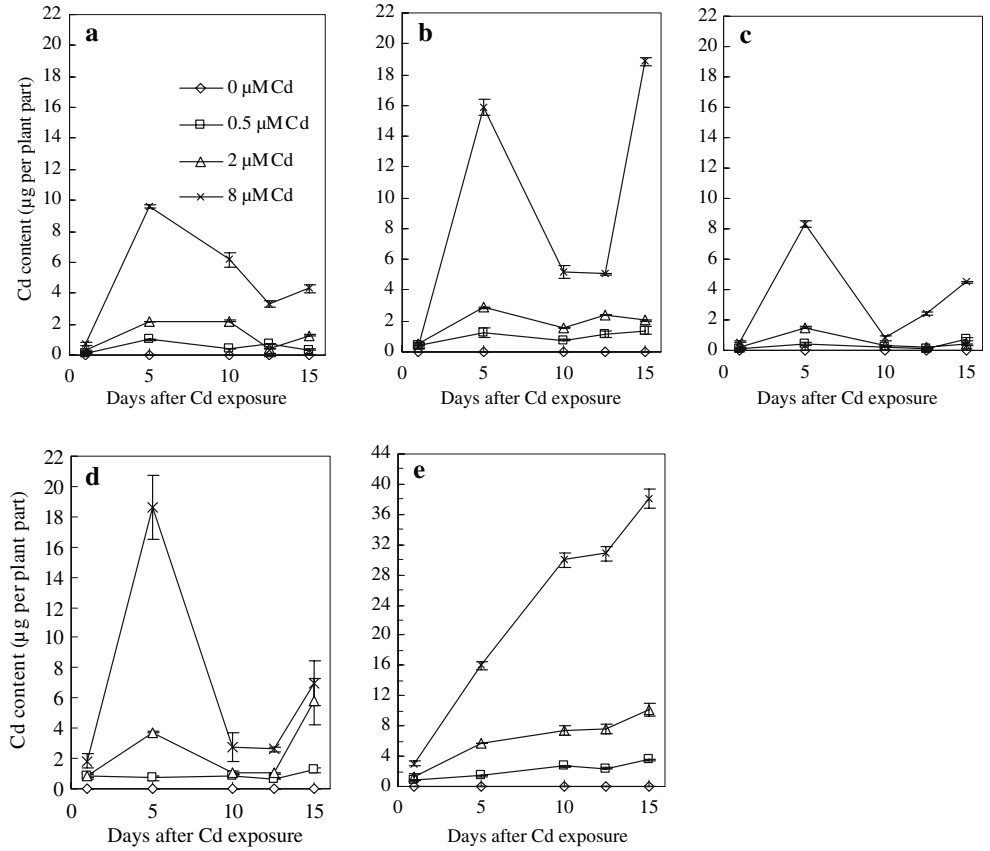

Fig. 2 Kinetics of per ear-based Cd accumulation of different part of barley shoots after 1, 5, 10, 12.5 and 15 days cultured under different Cd levels. Glume (**a**), awn (**b**), rachis (**c**), stem (**d**) and grain (**e**). Mean ± SD, $n = 3$

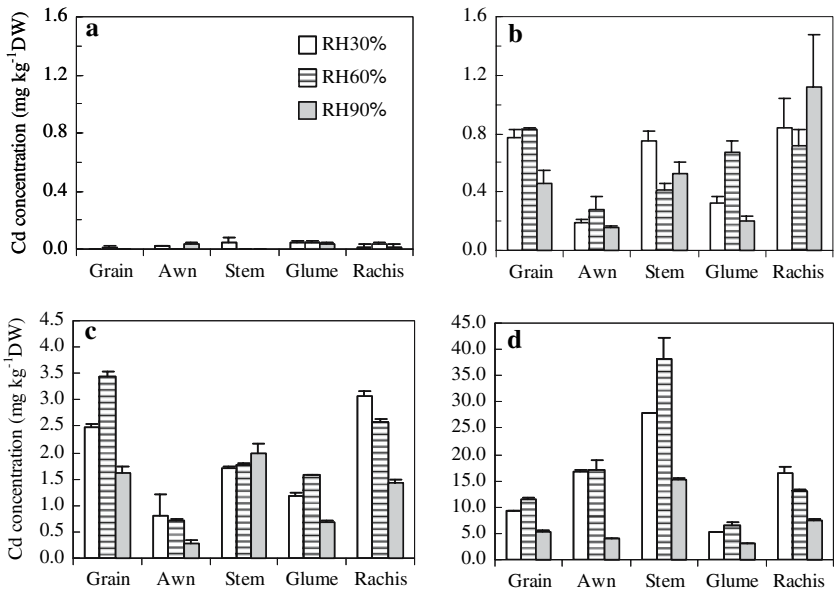

Fig. 3 Effect of relative humidity on Cd uptake and distribution in developing barley spikes and as affected by Cd levels. About 0 (**a**), 0.5 (**b**), 2 (**c**) and 8 (**d**) μM Cd for 5 days. Mean ± SD, $n = 3$

Effect of awn removal and stem-girdling on Cd uptake and distribution

Awn-removal and stem-girdling markedly decreased Cd concentration in grains, and stems of the shoots exposed to 2 and 8 μM Cd, especially for the spikes treated with awn-removal (Fig. 4). For instance, on average of the two Cd treatments, the reduction of Cd concentration caused by awn-removal and stem-girdling was 58.3, 38.2% in grains, and 55.1, 18.6% in stems, respectively, compared with the

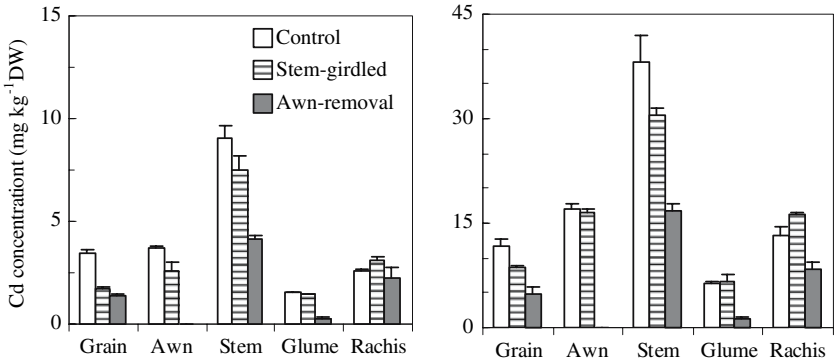

Fig. 4 Effect of awn removal and stem-girdling on Cd uptake and distribution in detached barley shoots subjected to 2 (*left*) and 8 (*right*) μM Cd for 5 days. Mean ± SD, $n = 3$

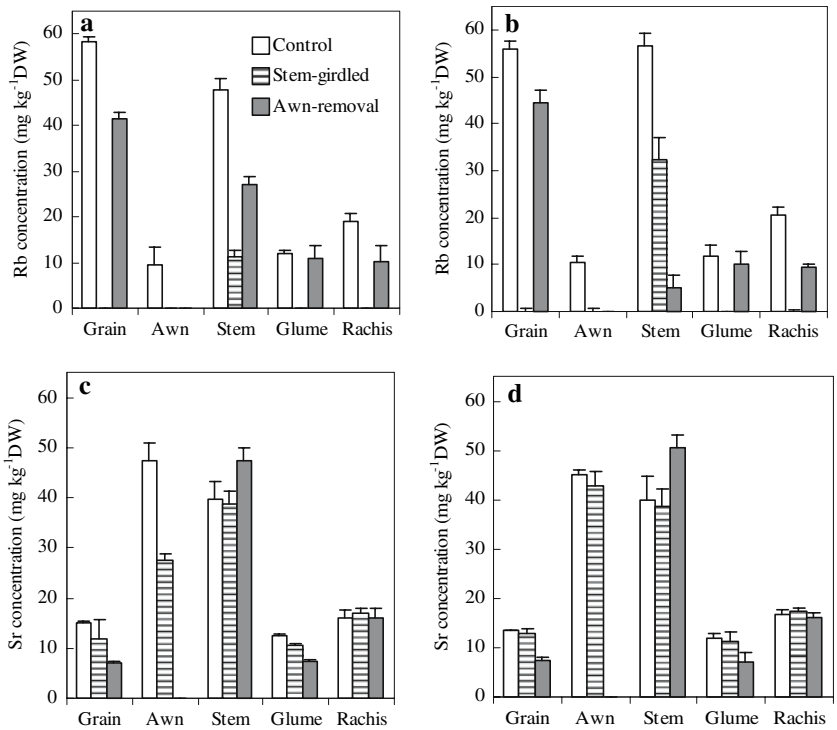

Fig. 5 Distribution of Rb (**a**, **b**) and Sr (**c**, **d**) in detached shoots cultured in 2 μM (*left*) and 8 μM Cd solution (*right*) for 5 days and as affected by awn removal and stem-girdling. Mean ± SD, $n = 3$

control. Awn removal almost blocked the transport of Cd to the glume and significantly restricted it to rachis in 8 μM Cd treatment, while stem-girdling had no effect on the former and even promoted the transport of Cd to rachis (cf. 19.4, and 23.0% of 2 and 8 μM Cd treatments, respectively, higher in Cd concentration over the control, $P < 0.05$). Transport of Cd to awns was reduced by stem-girdling in 2 μM Cd treatment, but no significant difference was found in 8 μM Cd treatment (Fig. 4).

Strontium was added to the nutrient solution as the marker of xylem transport, and less Sr was detected in grains and glumes in both 2 and 8 μM Cd treatments after awn-removal (Fig. 5c, d), compared with controls. Contrarily, Sr level significantly increased in stems of 2 and 8 μM Cd treatments (19.3 and 26.2%, respectively), but we observed no effects on rachis. In addition, awn Sr concentration of the stem-girdled shoots subjected to 2 μM Cd was 42.2% significantly lower than the control, while no significant difference in Sr concentrations was found in the other four parts of the spikes after stem-girdling (Fig. 5c, d). Almost no Rb was detected in grain, awn, glume and rachis after stem-girdling, and even stem had a lower concentration relative to control (Fig. 5a, b). Less Rb was observed in each part of detached shoots, except in glumes, after awn removal compared to the control (Fig. 5a, b).

Effect of sucrose level on Cd uptake and distribution

The addition of sucrose to the medium containing 2 μM Cd reduced Cd accumulation in grains and the other parts of shoots, and the effect strengthened with sucrose level in the medium (Fig. 6). That is, the Cd concentration in grains, stems, awns, glumes and rachides of detached shoots reduced by 50.0, 16.7, 77.0, 49.6, 24.3% in 30 mM, and by 92.4, 57.0, 85.1, 85.6, 72.7% in 150 mM sucrose addition, respectively, when compared with the controls (sucrose-free

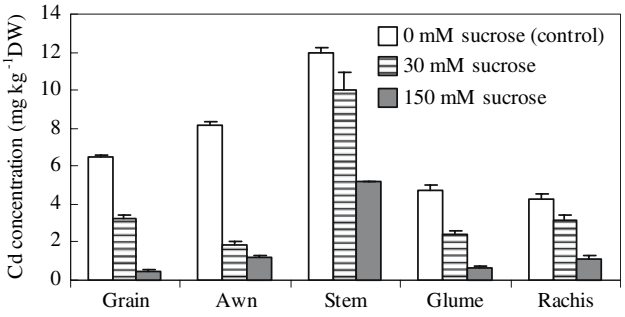

Fig. 6 Effect of sucrose status on Cd uptake and distribution in detached barley shoots subjected to 2 μM Cd for 5 days. Mean ± SD, $n = 3$

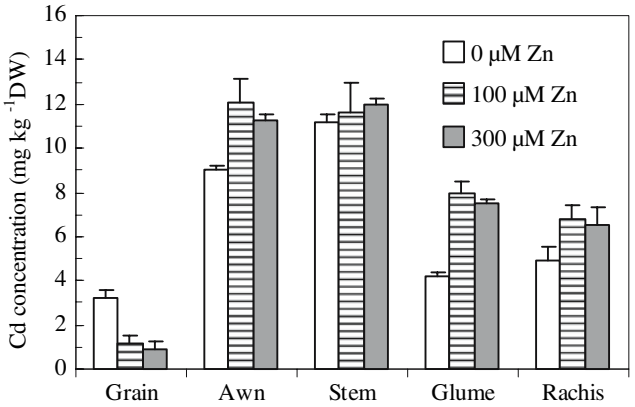

Fig. 8 Effect of Zn on Cd uptake and distribution in detached barley shoots subjected to 2 μM Cd for 5 days. Mean ± SD, $n = 3$

nutrient solution and the spikes kept in the dark for 24 h prior to sucrose/Cd added). Similarly, sucrose addition also largely reduced the transport of Rb and Sr to each part of the detached shoots (Fig. 7). Only little Rb and Sr were transported into spikes under 30 mM sucrose addition and even smaller amount was detected when the sucrose concentration was increased to 150 mM.

Effect of Zn addition on Cd uptake and distribution

The addition of 100, and 300 μM Zn in 2 μM Cd stressed shoots greatly decreased Cd concentration in grains by 63.0 and 72.9%, respectively, compared with the control (in the absence of Zn; Fig. 8). In contrast, Zn addition increased Cd concentration by 85.2, 28.8, 36.1 and 5.5% averaged over the two Zn treatments in glumes, awns, rachides and stems relative to control. Furthermore, Cd concentration in awns was higher in 100 μM Zn treatment than that in 300 μM Zn treatment and control (Fig. 8).

Discussion

Understanding the mechanism of Cd accumulation and translocation is an important area of research for safe food production. There are a few reports on Cd transport into wheat grains, e.g. Herren and Feller (1997) investigated the retranslocation of Cd via long-distance transport system and as affected by Zn application in wheat shoots exposed to 0.1 μM $CdCl_2$. Harris and Taylor (2001, 2004) examined the remobilization of ^{109}Cd applied to stem and flag in two near-isogenic wheat lines fed 1 ml of 250 pM ^{109}Cd. Based on the response of different parts of barley shoots to the Cd dose and exposure time and the effect of surrounding RH, awn removal and the addition of Zn/sucrose on Cd concentration/accumulation in the different parts of detached spikes, our results demonstrated that Cd accumulation in grains increased with external Cd levels and the time of exposure (Fig. 2). The increase of grain Cd accumulation in 10–12.5-day exposure over the 5-day exposure, with its relatively steady Cd concentration (Fig. 1), was paralleled with the decrease in Cd content of the other four parts of detached shoots (Fig. 2), with the exception of rachis Cd content at day 12.5 (i.e. higher than that at day 10 but still lower than that at day 5). This finding indicates substantial remobilization of Cd into grains from other plant parts, and suggests that awn, rachis and glume may be involved in Cd transport into the developing grains, especially in the grain filling stage, and that the redistribution of Cd in maturing spikes must be considered as an important physiological process influencing final product quality. Consequently, the transport from vegetative plant parts to the spikes and even from floral organs to grains can be relevant for Cd accumulation, and may pose a negative impact on yield formation

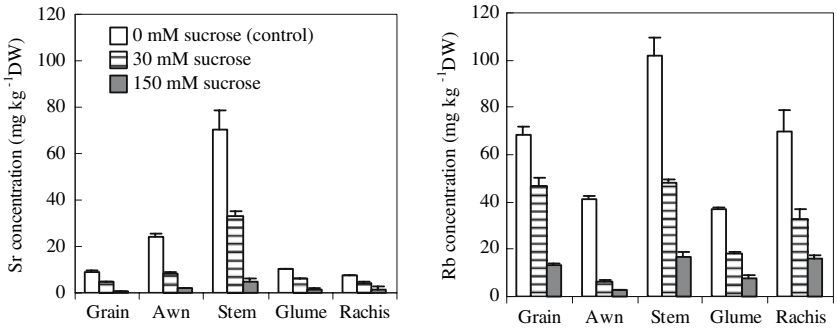

Fig. 7 Effect of sucrose status on Sr (*left*) and Rb (*right*) uptake and distribution in detached barley shoots subjected to 2 μM Cd for 5 days. Mean ± SD, $n = 3$

under certain environmental conditions (e.g. Cd contamination). On the other hand, a clear increase in Cd content in all five spike parts were observed from 12.5 to 15-day exposure. This might indicate that Cd continued to accumulate in all floral parts of the spike, and that the remobilization of Cd from other parts to grains was reduced because grain filling slowed down. However, further studies are needed to clarify the mechanism of Cd retranslocation to grains.

Marschner (1986) suggested that, with plant development, transpiration rates might become increasingly important for Cd absorption and translocation. Reduced transpiration is also thought to block translocation within the xylem and the xylem-to-phloem transfer in the spike and to lightly affect the phloem transport to the grains (Pearson et al. 1996). In the present study, high RH (90%) treatment, which should reduce transpiration relative to the other two lower RH (30 and 60%), resulted in a significant decrease in Cd concentrations in grains, awns and glumes of the spikes exposed to various Cd stresses (Fig. 3). It could be suggested that Cd transport was markedly affected by the transpiration and xylem transport. High RH may also reduce phloem transport into grains (Pearson et al. 1996) due to the increase in water potential resulting from reduced water loss of the grain, although this effect may be small. On the other hand, it is interesting to observe that low grain Cd concentration recorded in 30% RH, irrespectively of Cd levels, with the highest concentration in rachides and awns (Fig. 3), compared with that in 60% RH. Whether the xylem-to-phloem transfer in the spikes was reduced by the excess water loss in low RH (30%), and a large part of Cd was remained in the xylem stream and accumulated finally in the rachides and awns needs to be studied in detail.

Pearson et al. (1996) reported that awn removal reduced the transport of Zn and Mn into lemma, but not into grains. In the present study, awn removal caused a significant decrease in Cd transport into the spikelet (Fig. 4). Awns are important in evaporative water loss and movement of xylem sap containing Cd is reduced when awns are not present. Thus, awns represent structures important in transpiration-driven xylem transport to spikes. On the other hand, essentially no Rb was detected in spikes of the detached shoots while the Sr content remained more or less at the same level as the control after stem-girdling (Fig. 5). This proves that stem-girdled plants had only their phloem transport blocked, while xylem transport was functioning (Jenner 1985; Haslett et al. 2001). A reduction in grain Cd concentration was observed as the phloem transport blocked via stem-girdled (Fig. 4), consistent with the results of Herren and Feller (1997), indicating that Cd transport into spikes and especially in the grains partially depended on or chaperoned by phloem transport. Rachis Cd concentration, in contrast, increased by stem-girdled but decreased by awn-removal, implying its dominant mechanism of xylem transport.

We also investigated the importance of mass flow and energy dependence on Cd transport by varying the sucrose status in the culture solution. Increasing sucrose levels in the solution induced a strong reduction in Cd content in grains and the other parts of detached shoots (Fig. 6), and also largely reduced the transport of Rb and Sr to spikes (Fig. 7). This indicated that phloem and xylem transport were reduced by high sucrose content in the spikes, which would explain the observed reduction in Cd transport to the developing spikes. Large amount of sucrose added in the culture solution may led to the accumulation of sucrose in the spikelet as phloem loading would become saturated due to the saturation of membrane transporters as Pearson et al. (1996) suggested. In addition, increasing sucrose level would result in reduced water absorption due to decreased water potential and higher viscosity of the feeding solution, which may slower both the xylem and phloem transport and reduce Rb and Cd, Sr translocation.

The addition of Zn in the nutrient solution greatly reduced Cd transport into the grains (Fig. 8), while increased Cd concentrations in the other four parts of shoots. Cd and Zn may compete for the same loading sites in the xylem-to-phloem transfer. As a consequence, a considerable amount of Cd could not be transferred into the phloem and less Cd could be transferred from stem or remobilized from the awn, glume and rachis as the grain matures. Thus, relatively more Cd retained in awn, glume and rachis. Otherwise a high concentration of Zn has toxic effects on the plants, as the dry weight of the grain reduced by 22.5% in 300 μM Zn treatments compared to the control (Zn absent) in our experiments. The toxic effects may reduce mass flow of phloem (Rauser and Samarakoon 1980) or induce the saturation of membrane transporters (Pearson et al. 1996), which may impede Cd translocation to the grains. The well known capacity of plants to accumulate Cd in grains or seeds to higher amounts under Zn-deficient conditions (Moraghan 1993; Oliver et al. 1994) can also be attributable to Zn-deficiency induced phloem-mediated transport of Cd into seeds or grains from source organs, i.e. mature roots and mature leaves. This finding suggests that one strategy for reducing grain Cd concentration is to improve Zn nutritional status of plants via soil or foliar applications, particularly during reproductive growth.

In conclusion, Cd transports from stems to ears, chaperoned by phloem transport, mainly via xylem transport and phloem transport plays a significant role in the translocation of Cd into the developing barley grains. The floral parts of spikes such as awn, rachis and glume likely are involved in Cd transport into the developing grains, and the redistribution of Cd in maturing barley plants should be considered as an important physiological process influencing

final grain quality. Environment conditions around the developing spikes greatly affect the transport of Cd to the developing grains. Addition of sucrose in the cultural medium induced a strong reduction of Cd transport to developing grains of the detached shoots. Increasing the RH to 90% and addition of Zn in the medium at the grain filling stage would be beneficial to decrease Cd accumulation in barley grains.

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