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RESEARCH ARTICLE

Accumulation of cadmium in near-isogenic lines of durum wheat (*Triticum turgidum* L. var durum): the role of transpiration

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Abstract Concentrations of cadmium in the grain of durum wheat (*Triticum turgidum* L. var durum) are often above the internationally acceptable limit of 0.2 mg kg^{-1}. Cultivars that vary in concentrations of cadmium in the grain have been identified but the physiology behind differential accumulation has not been determined. Three pairs of near-isogenic lines (isolines) of durum wheat that vary in aboveground cadmium accumulation (8982-TL 'high' and 'low', W9260-BC 'high' and 'low', and W9261-BG 'high' and 'low') were used to test the hypothesis that the greater amounts of cadmium in shoots of the 'high' isolines are correlated with greater volumes of water transpired. In general, cadmium content was positively correlated with transpiration only in the 'low' isolines. Although shoots of the 'high' isolines of W9260-BC and W9261-BG contained higher concentrations of cadmium than did their corresponding 'low' isolines, they did not transpire larger volumes of water. In addition, isolines of 8982-TL transpired less water than did the other pairs of isolines yet both 'high' and 'low' isolines of 8982-TL contained higher amounts of cadmium than did the other pairs. The difference between 'high' and 'low' isolines appears to be related to the relative contribution of transpiration to cadmium translocation to the shoot. Increased transpiration was associated with increased cadmium content in the 'low' isolines but in the 'high' isolines increased cadmium in the shoot occurred independently of the volume of water transpired.

Keywords Cadmium · Durum wheat · Near-isogenic lines · Transpiration · *Triticum turgidum*

Introduction

Cadmium is a nonessential element that has the ability to form strong complexes with biomolecules (Bolton and Evans 1996), making it potentially harmful in small quantities. The effects of cadmium on plants include reduced growth, inhibition of photosynthesis (Weigel 1985) and changes in stomatal action that affect water relations (Barcelo et al. 1988). Because cadmium can cause a number of adverse effects in humans (reviewed in Bernard and Lauwreys 1984), the Codex Alimentarius Commission (CAC), which was established jointly by the Food and Agriculture Organization and World Health Organization, has set international food standards to help ensure food safety. The limit for cadmium in wheat (*Triticum* spp) is currently 0.2 mg kg^{-1} (CAC 2010). As an added measure, some countries have established guidelines above which soils should not be used for food crops. For example, in Canada, soils that have a concentration of cadmium above 1.4 mg kg^{-1} dry weight are considered to be unsafe for agriculture (CCME 1999). In the United Kingdom, the upper limit for cadmium in allotment soil (i.e. soil used for municipal and home gardens) is 1.8 mg kg^{-1} dry weight (EA 2009).

Concerns have been raised due to increasing concentrations of cadmium in agricultural soils (cf. Williams and David 1976)

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and the propensity of certain crops, including durum wheat (*Triticum turgidum* L. var durum), to accumulate cadmium (reviewed in Grant et al. 2008). Durum wheat grains grown in Canada (Garrett et al. 1998) and the USA (Zook et al. 1970) sometimes exceed the international cadmium standard of 0.2 mg kg^{-1}; however, the concentrations of cadmium in the grain can vary up to six-fold, depending on cultivar, location and year (Clarke et al. 2002). Contamination levels in crops could be reduced by means of chemical remediation (Makino et al. 2008) or phytoremediation of the soil (Ishikawa et al. 2006; Murakami et al. 2007) as well as adjusting agronomic practices to avoid soil and field sites in which cadmium mobility is high (Cieslinski et al. 1996; Wu et al. 2002). Another approach to reducing cadmium in the crop, which has been applied to durum wheat, is through the selection of cultivars or lines that accumulate less cadmium. For example, Clarke et al. (1997a) derived five pairs of near-isogenic lines (isolines) of durum wheat (Table 1) by crossing different combinations of parental lines that are relatively 'low' or 'high' cadmium accumulators. The isolines were selected in agricultural soils that contained <1.4 mg Cd kg^{-1}.

Explaining differential cadmium translocation in durum wheat

Accumulation of cadmium in the grain is controlled by a single gene, dominant for low cadmium-accumulation (Clarke et al. 1997b). Recently, Knox et al. (2009) mapped the locus of the gene for cadmium-accumulation (*Cdu1*) on chromosome 5B. The corresponding physiology behind differential cadmium translocation in durum wheat has not yet been elucidated.

A number of characteristics related to metal uptake and translocation have been studied using cultivars of durum wheat that vary in the amounts of cadmium in their shoots and grain. The two most commonly studied have been Kyle (a 'high' accumulator) and Arcola (a 'low' accumulator). The amount of cadmium in the shoot and grain in the cultivar Kyle can be as much as twice that of Arcola (Chan and Hale 2004). Cieslinski et al. (1998) reported that root exudates of Kyle contained higher concentrations of low molecular weight organic acids than did exudates of Arcola. They hypothesized that this might result in increased solubility of metals in the rhizosphere of Kyle, leading to increased uptake of cadmium from the soil. Berkelaar and Hale (2000) found a positive relationship between accumulation of cadmium by roots and number of root tips; Kyle had a smaller root surface area, fewer root tips and 35 % less cadmium in the roots than did Arcola. This result seems counterintuitive, since one might expect the 'high' accumulator to take up more cadmium. However, others have also reported that 'high' accumulators do not necessarily take up more cadmium per plant; thus, while 'high' accumulators have higher concentrations of cadmium in the leaves and grain, they often have lower concentrations of cadmium in the roots (Chan and Hale 2004; Greger and Lofstedt 2004; Hart et al. 2006; Van der Vliet et al. 2007).

Once cadmium is taken up by a plant, the transpiration stream can carry it in the xylem from the roots into the aboveground tissues. Concentrations of cadmium in grains of barley (*Hordeum vulgare* L.) were 40–50 % lower in plants grown at 90 % relative humidity than for plants grown at 60 % or 30 % relative humidity, presumably due to lower transpiration rates at the higher relative humidity (Chen et al. 2007). Van der Vliet et al. (2007) examined a number of responses to cadmium in the 'high' accumulating cultivar Kyle and the 'low' accumulating cultivar Arcola; exposure to cadmium induced an increase in the transpiration rate for Kyle, but not Arcola. This suggests that factors related to transpiration differ between 'high' and 'low' cadmium-accumulators.

While studies of cultivar pairs, such as Kyle and Arcola, provide important information about cadmium/plant interactions, the large number of genetic and physiological differences between cultivars makes it difficult to pinpoint the mechanism (s) behind 'high' and 'low' accumulators. For this reason, Clarke et al.'s (1997a) isolines of durum wheat provide an excellent experimental model system for investigating the physiology underlying differential accumulation of cadmium. The main difference between Clarke et al.'s (1997a) 'high'

Table 1 Five pairs of near-isogenic durum wheat derived by Clarke et al. (1997a). The letter following the cultivar name indicates a 'low' cadmium accumulator (L) or a 'high' cadmium accumulator (H)

Cultivar Name	Genetic Stock, Registration Number	Derived from crossing
8982-SF-(L)	GS-81, PI591058	Kyle/Nile
8982-SF-(H)	GS-82, PI591059	
8982-TL-(L)	GS-83, PI591060	Kyle/Nile
8982-TL-(H)	GS-84, PI591061	
W9260-BC-(L)	GS-85, PI591062	DT61/DT471
W9260-BC-(H)	GS-86, PI591063	
W9261-BG-(L)	GS-87, PI591064	DT630/DT471
W9261-BG-(H)	GS-88, PI591065	
W9262-339A-(L)	GS-89, PI591066	Kyle/Biodur
W9262-339A-(H)	GS-90, PI591067	

and ‘low’ isolines is in the distribution of cadmium within the plant — relatively more cadmium is translocated from root to shoot and grain in the ‘high’ isolines. It follows that the physiological differences between the ‘high’ and ‘low’ isolines are related to the differential distribution of cadmium.

In an attempt to determine if the ‘high’ and ‘low’ isolines of durum wheat differ with respect to metal-binding molecules, both phytochelatins (Hart et al. 2006) and organic acids (Adeniji et al. 2010) have been studied. In each case, no clear relationship between translocation of cadmium in the shoot and concentration of metal-binding molecules was found. Given the expectation that increased transpiration could result in increased accumulation of cadmium in the plant in general, and in the aboveground tissues in particular, this experiment was designed to test the following hypotheses: (1) the accumulation of cadmium in the aboveground tissues is positively correlated with the volume of water transpired and (2) the ‘high’ isolines of durum wheat have higher transpiration rates than do the ‘low’ isolines.

Materials and methods

Choice of experimental lines

Three pairs of Clarke et al.’s (1997a) isolines of durum wheat (*Triticum turgidum* L. var durum; 8982-TL ‘high’ and ‘low’, W9260-BC ‘high’ and ‘low’, and W9261-BG ‘high’ and ‘low’) were chosen for study. This choice was based on Bahrami (2006), who demonstrated that differential accumulation of cadmium in the ‘high’ and ‘low’ members of these pairs could be obtained in hydroponic culture. The other two pairs of isolines were not included because neither Bahrami (2006) nor Macfie (unpublished data) were able to reproduce differential cadmium accumulation in the ‘high’ and ‘low’ isolines of these pairs when grown hydroponically, despite repeated attempts. Seeds were obtained from Dr. John Clarke (Agriculture and Agri-Food Canada, Swift Current, SK).

Germination and growth conditions

Modifications of Archambault et al.’s (2001) method were used to grow the durum wheat. The seeds were surface-sterilized for 20 min in 1 % sodium hypochlorite (Javex), rinsed three times with distilled water, and placed in an aerated solution containing 0.005 gL^{-1} Vitavax (a systemic fungicide; Uniroyal Chemical Ltd., Calgary, Canada) for 24 h. The flasks were covered with foil to prevent light from reaching the seeds and Parafilm was placed over the top of the flasks to prevent evaporation of the solution. After the seeds germinated (~24 h) they were sown at 3–5 mm depth in a pot filled with coarse sand (1–2 mm grain size). Pots were watered with nutrient solution containing 1.0 mM $Ca(NO_3)_2$, 1.0 mM K_2HPO_4, 0.4 mM KNO_3, 0.3 mM NH_4NO_3, 0.1 mM K_2SO_4, 0.01 mM $FeCl_3$, 0.01 mM Na_2EDTA, 6.0 μM H_3BO_3, 2.0 μM $MnCl_2$, 0.5 μM $ZnSO_4$, 0.15 μM $CuSO_4$, and 0.1 μM Na_2MoO_4, adjusted to pH 6. Pots were placed in a controlled environment room set to 20 °C with a 16 h light period and an 8 h dark period; the fluorescent light intensity was 187 ± 1.5 μmol m^{-2} s^{-1}.

After 1 week, seedlings of uniform size were selected for hydroponic culture experiments. Groups of five seedlings were suspended in folded upholsterer’s foam (approximately $0.5\times1.0\times8$ cm) and placed in a slot cut into the black lid of a 1.4 L glass jar. Each treatment had six replicates. The jars were covered in black cloth to prevent algal growth and were filled with 1.4 L nutrient solution containing 0 or 0.1 μM $CdCl_2$ adjusted to pH 6. Cadmium concentrations greater than 0.1 μM have been demonstrated to cause stress in durum wheat (Archambault et al. 2001) and when investigating differential cadmium accumulation patterns it is important that the plants do not experience cadmium stress. Each jar was hooked up to an aeration system.

Experimental measurements

Each day, the length of the longest shoot in each jar was taken as a measure of growth and the mass of each jar was recorded to measure water loss. Evaporation from a set of three jars filled with distilled water was negligible over the experimental period (0.020 ± 0.001 ml per week). Therefore, mass lost by each jar was deemed to be equal to the mass of water lost through transpiration. The nutrient solution (including the corresponding cadmium treatment) was fully replaced in each jar every second day to ensure adequate nutrition for the growing plants.

Twelve days after the cadmium was added, the plants were harvested; the five seedlings per jar were pooled to represent one replicate sample. For each sample, the shoots were separated from the roots using a razor blade, and root and shoot fresh weight as well as total leaf area were measured. Cadmium concentrations in the shoots of seedlings are a good predictor of the cadmium concentrations of the grain (Archambault et al. 2001), and were used to evaluate the relative ‘high’ versus ‘low’ classification of the isolines. The roots were rinsed in distilled water for 30 s then placed in 1 mM $CaCl_2$ for 30 min followed by another 30 s rinse in distilled water (Taylor et al. 1998). This procedure removes cadmium adsorbed to the root surface by means of a cation exchange reaction between Cd^{2+} and Ca^{2+}. All tissues were oven-dried (60 °C) to constant weight (5–6 days) then dry weight was recorded. Dry portions were processed for cadmium content.

Cadmium content

Three samples of seeds (approx. 20–25 seeds each) of each isoline were homogenized in a mechanical grinder and processed to determine their initial concentrations of cadmium. Ground seeds were oven-dried (60 °C) to constant weight, then 1 g of each sample was placed into individual 15 mL test tubes; 2.0 mL of nitric acid (OmniTrace®) was added to each test tube to digest the seeds. Dried plant tissues were finely chopped using a razor blade and approximately 0.1 g of each sample (shoots or roots) were weighed into individual 15 mL test tubes; 1.0 mL of nitric acid (OmniTrace®) was added to each test tube to digest the tissues. Reagent blanks and tomato leaves (NIST Standard Reference Material 1573a, National Institute of Standards and Technology, Gaithersburg, USA) were processed similarly to aid in determining possible contamination and percentage recovery of cadmium in the tissue samples, respectively. Each test tube was caped with a clean marble to ensure the sample remained in the test tube and allowed for pressure release. The test tubes were placed in a rack and left overnight at room temperature under a fume hood. The rack of test tubes was then placed in a tray filled with a 3–4 cm depth of fine sand (0.2–0.5 grain size) and heated to 95–100 °C on a hot plate until tissues were completely digested. Samples were considered to be completely digested when the vapours became colourless. When samples returned to room temperature, they were filtered (qualitative paper #413, VWR International, Mississauga, Canada) into sterile disposal centrifuge tubes. Deionized water was used to rinse the test tubes and bring the volume up to 25 mL.

Samples were analyzed for cadmium content using an inductivity-coupled plasma optical emission spectrometer (ICP-OES) using the following conditions: Perkin-Elmer Optima 3300 dual view ICP-OES, with a RF generator power of 1,300 W; gas flow rate: 15 $\mathrm{L min^{-1}}$: auxiliary flow rate: 0.5 $\mathrm{L min^{-1}}$; nebulizer flow rate: 0.8 $\mathrm{L min^{-1}}$; pump (for sample) flow rate:1.0 $\mathrm{L min^{-1}}$; analyte line: Cd, 226.507 nm; with a detection limit of 0.0010 mg $\mathrm{L^{-1}}$ for cadmium. Duplicate samples and calibration standards were run every 10–15th sample to verify instrument accuracy.

Statistical analysis

Statistical analysis was performed using SigmaPlot version 11. If data did not meet the requirements of normality or homogeneity of variance, the data were transformed prior to analysis. Two-way analysis of variance (ANOVA) was performed to compare the initial concentrations of cadmium in the seeds, as well as to determine the effects due to isoline and cadmium treatment on each of plant biomass, cadmium content and total volume of water transpired. The Tukey's test was used to determine the significant differences among means ($p<0.05$). Two-way repeated measures ANOVA were performed to determine the effects of time and cadmium treatment, and the effects of time and isoline, on the maximum shoot length as well as the cumulative daily transpiration by plants. Once again the Tukey's test was used to determine the significant differences among means.

Results

Effect of cadmium on plant growth

Cadmium affected neither shoot nor root dry weight in the isolines 8982-TL ($p>0.43$) and W9260-BC ($p>0.49$); the root dry weight of the two isolines of W9621-BG were similarly unaffected by cadmium ($p=0.06$; Fig. 1). However, the dry weights of the shoots of both 'high' and 'low' isolines of W9621-BG were reduced by approximately 20 % when exposed to cadmium (Fig. 1). All plants grew over the course of the experiment and appeared healthy. While cadmium did not affect shoot length ($p>0.29$), the 'high' isolines were 1–1.5 cm (approx. 3 %) shorter than the corresponding 'low' isolines at the end of the experimental period (data not shown).

Cadmium content

The initial concentrations of cadmium in seeds confirmed the expected pattern: concentrations of cadmium in seeds of the 'high' isolines were 1.6–2.4 times higher than those of the corresponding 'low' isolines' (Table 2). Concentrations

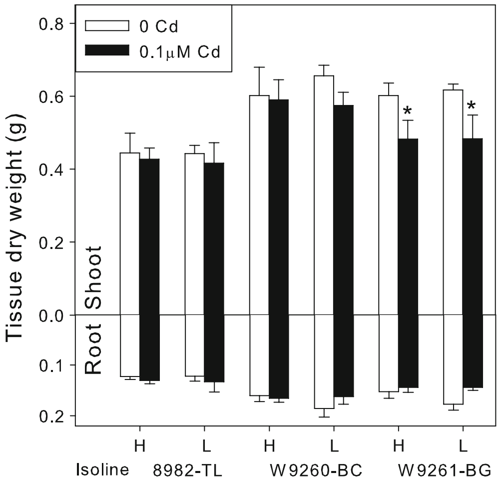

Fig. 1 Dry weight of shoots and roots of three pairs of isolines of durum wheat. Within each pair, H denotes the 'high' cadmium-accumulating isoline and L denotes the 'low' cadmium-accumulating isoline. Plants were harvested after 12 days growth in nutrient solution (*white*) or nutrient solution with 0.1 μM $\mathrm{CdCl_2}$ (*black*). Bars represent SE of six replicates. Asterisks indicate a significant difference between control and cadmium-treated plants within an isoline

Table 2 Concentrations of cadmium in the seeds of near-isogenic durum wheat. The measurements were taken from seed samples collected prior to the start of the experiment. Significant differences ($p<0.05$) in cadmium concentrations among the isolines are indicated by different lower case letters

Cultivar name	Concentration (+ SE) of cadmium in 'high' isolines (μg g^{-1})	Concentration (+ SE) of cadmium in 'low' isolines (μg g^{-1})
8982-TL	0.280±0.008a	0.110±0.005c
W9261-BC	0.217±0.001b	0.131±0.001c
W9261-BG	0.195±0.002b	0.093±0.003c

of cadmium in shoots and roots of the isolines are shown in Fig. 2. Trace amounts of cadmium were measured in tissues of plants grown in control solution. Within the cadmium-treated plants, the concentrations of cadmium were 5–6 times higher in roots than in shoots. When the isolines of 8982-TL were grown with 0.1 μM cadmium, there were no differences in concentrations of cadmium in the shoots (p=0.90) or roots (p=0.57) of the 'high' isoline compared to those of the 'low' isoline. Within the shoots of the isoline pairs of W9260-BC and W9261-BG, the 'high' isolines had 15–20 % higher cadmium concentrations compared to the 'low' isolines. The roots of the 'low' isoline of W9260-BC had a 25 % higher concentration of cadmium compared to the 'high' isoline; the concentrations of cadmium in roots of the 'high' and 'low' isolines of W9261-BG did not differ (p=0.76).

Effect of cadmium on transpiration

Daily transpiration per plant increased with time (Fig. 3a). As assessed by repeated measures ANOVA, the daily cumulative transpiration did not differ between plants grown under different cadmium treatments (p=0.35) but it did differ among isolines. Over the course of the experiment, the daily transpiration volumes of the 'high' and 'low' isolines of

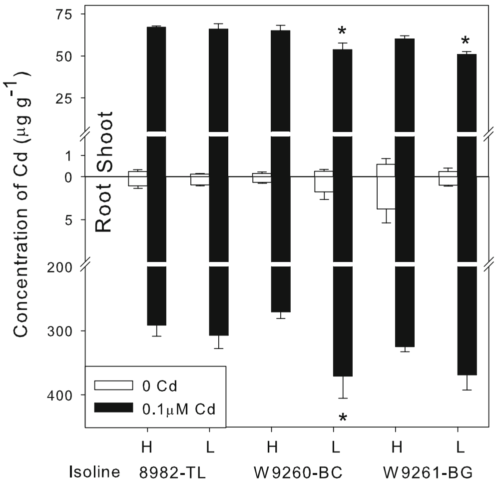

Fig. 2 Concentration of cadmium in shoots and roots of three pairs of isolines of durum wheat. Within each pair, H denotes the 'high' cadmium-accumulating isoline and L denotes the 'low' cadmium-accumulating isoline. Plants were harvested after 12 days growth in nutrient solution (*white*) or nutrient solution with 0.1 μM $CdCl_2$ (*black*). *Bars* represent SE of six replicates. Asterisks indicate a significant difference between 'high' and 'low' pairs of isolines

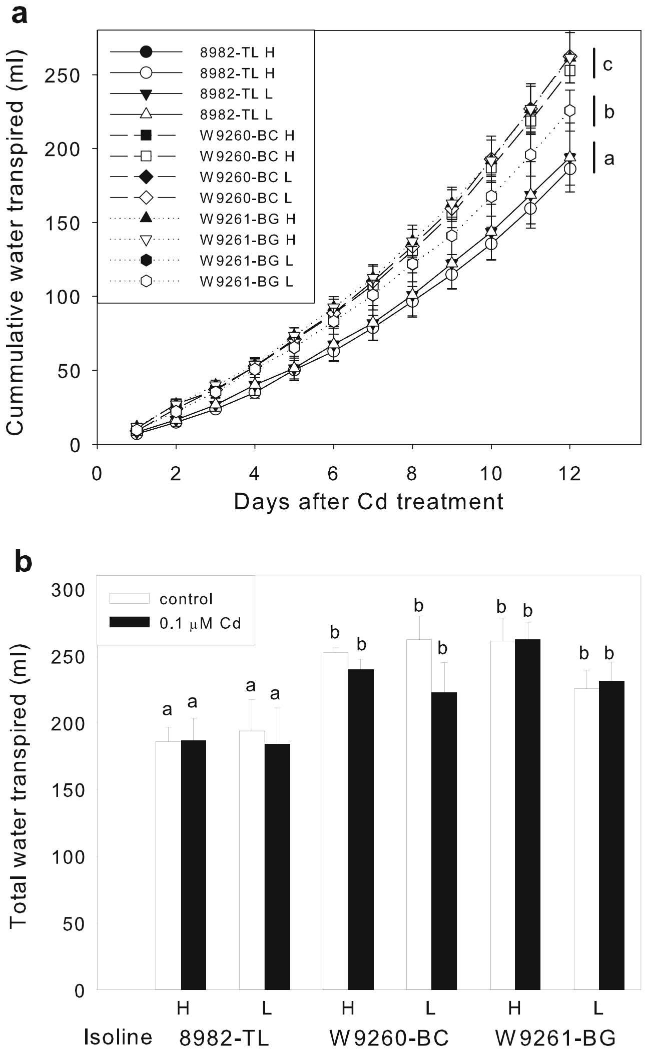

Fig. 3 Cumulative daily volume of water transpired (**a**) and total volume of water transpired (**b**) by three pairs of isolines of durum wheat. Within each pair, H denotes the 'high' cadmium-accumulating isoline and L denotes the 'low' cadmium-accumulating isoline. Plants were grown in nutrient solution (*white*) or nutrient solution with 0.1 μM $CdCl_2$ (*black*). Bars represent SE of six replicates. Lower case letters indicate significant differences in transpiration over the 12-day growth period (**a**) and significant differences in the total volume of water transpired (**b**)

8982-TL were lower than those of the other isolines, for both control and cadmium-treated plants. Intermediate volumes of water were transpired by cadmium-treated ‘low’ isolines of W9260-BC and W9261-BG, as well as the ‘low’ isoline of W9261-BG grown in control conditions. The remaining isolines transpired higher volumes of water. The total volume of water transpired per plant by day 12 was also assessed (Fig. 3b). By this measure, cadmium treatment did not affect the volume of water transpired (p=0.79), and isolines of 8982-TL transpired less water than did the other two pairs of isolines.

When transpiration was standardized based on leaf area, a significant difference was found between cadmium-treated and control plants for isoline W9261-BG only (Fig. 4). Cadmium treated plants had 35 % and 16 % higher transpiration rates for W9261-BG ‘high’ and ‘low’ isolines, respectively.

Relationship between cadmium accumulation and transpiration

To determine the relationship between transpiration and cadmium uptake, correlations between the total volume of water transpired per jar and the amount of cadmium in the plant samples were determined. Positive correlations were found between the total amount of water transpired and the total cadmium content for each of the ‘low’ isolines, whereas no correlations were observed for the ‘high isolines (Fig. 5a, Table 3). When only the amounts of cadmium in the shoots were considered, positive correlations between transpiration and cadmium content were observed only for the ‘low’ isolines of W9260-BC and W9261-BG (Fig. 5b, Table 3).

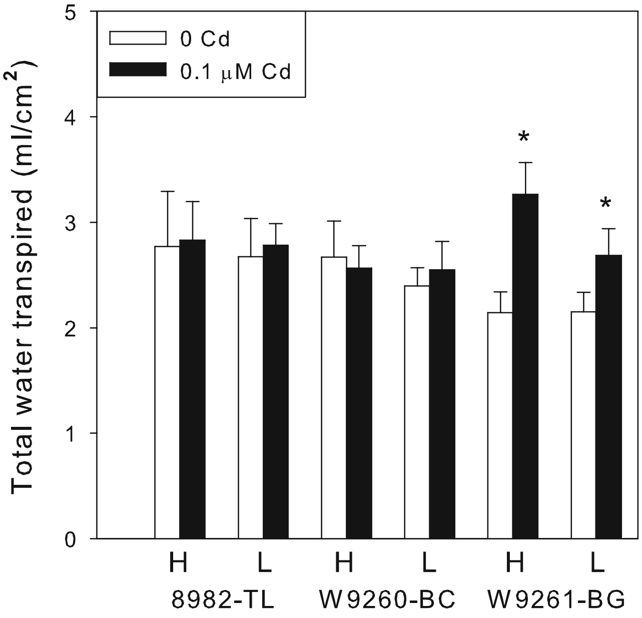

Fig. 4 Total volume of water transpired per unit leaf area for three pairs of isolines of durum wheat. Within each pair, H denotes the ‘high’ cadmium-accumulating isoline and L denotes the ‘low’ cadmium-accumulating isoline. Plants were harvested after 12 days growth in nutrient solution (*white*) or nutrient solution with 0.1 μM $CdCl_2$ (*black*). *Bars* represent SE of six replicates. Asterisks indicate a significant difference between control and cadmium-treated plants within an isoline

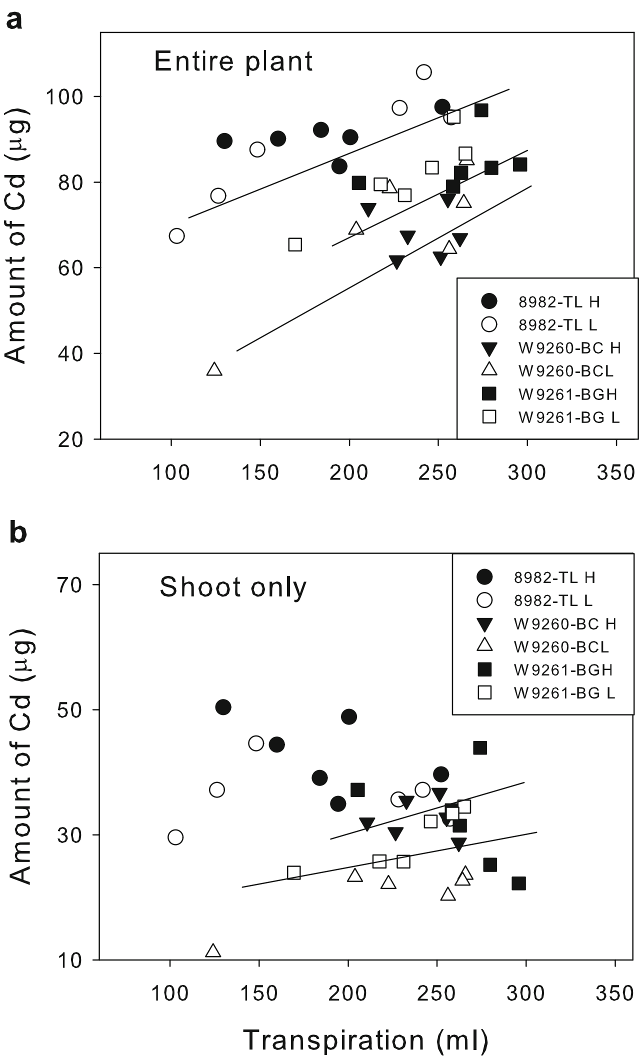

Fig. 5 Relationships between amount of cadmium in entire plant (**a**) or shoot only (**b**) and total volume of water transpired for three pairs of isolines of durum wheat. Within each pair, H denotes the ‘high’ cadmium-accumulating isoline (*black*) and L denotes the ‘low’ cadmium-accumulating isoline (*white*). Plants were grown for 12 days in nutrient solution with 0.1 μM $CdCl_2$. Regression lines indicate significant correlations (see Table 3)

Discussion

Isolines of durum wheat that differ only in the relative amounts of cadmium in aboveground tissues provide an opportunity to test theories about physiological controls over the distribution of cadmium among plant tissues. However,

Table 3 The Pearson Product Moment correlation coefficient (r) and the significance level (p) for the relationship between amount of cadmium and the volume of water transpired by each isoline grown in 0.1 μM $CdCl_2$. The letter following the cultivar name indicates a 'low' cadmium accumulator (L) or a 'high' cadmium accumulator (H). The top half of the table contains information for the amount of cadmium in the entire plant (corresponding to Fig. 5a); the bottom half contains information for the amount of cadmium in the shoot (corresponding to Fig. 5b). Significant correlations ($p<0.05$) are indicated in bold

Cultivar Name	r	p
Cd in entire plant		
8982-TL-(L)	**0.92**	**0.010**
8982-TL-(H)	0.51	0.304
W9260-BC-(L)	**0.86**	**0.027**
W9260-BC-(H)	−0.08	0.877
W9261-BG-(L)	**0.96**	**0.003**
W9261-BG-(H)	0.40	0.432
Cd in shoot		
8982-TL-(L)	−0.03	0.951
8982-TL-(H)	−0.52	0.291
W9260-BC-(L)	**0.84**	**0.035**
W9260-BC-(H)	−0.04	0.946
W9261-BG-(L)	**0.88**	**0.020**
W9261-BG-(H)	−0.50	0.318

the isolines derived by Clarke et al. (1997a) are not especially cadmium-tolerant. For example, Archambault et al. (2001) reported a 30 % reduction in root dry weight for isolines of 8982-TL grown in solution with only 0.5 μM $CdCl_2$. Nor do the isolines retain their characteristic 'high' or 'low' concentrations of cadmium in aboveground tissues when exposed to concentrations of cadmium in solution above 0.5 μM $CdCl_2$ (Macfie unpublished data). For these reasons, experiments designed to test physiological differences that might explain differential cadmium accumulation patterns in durum wheat must be done under conditions free of cadmium-stress. In this experiment, neither isolines of 8982-TL and W9260-BC had symptoms of cadmium-induced stress as measured by dry weight, shoot length and visible appearance. Despite a slight reduction in shoot biomass in cadmium-treated plants, the isolines of W9261-BG appeared similarly healthy.

Ideally, experiments designed to investigate the mechanisms behind 'high' and 'low' isolines should verify differential patterns of cadmium accumulation. Under this study's experimental conditions, only isolines W9260-BC and W9261-BG showed the expected greater cadmium concentration in the shoots of the 'high' isoline relative to the 'low' isoline, even though the initial concentrations of cadmium in the seeds of the 'high' isolines were consistently higher than those in the 'low' isolines for each pair, and were well within the ranges reported by Clarke et al. (2002). Bahrami (2006) grew all five pairs of Clarke et al.'s (1997a) isolines of durum wheat in solutions with 0.1 μM $CdCl_2$ for 12 days and reported higher concentrations of cadmium in the 'high' isolines of 8982-TL, W9260-BC and W9261-BG, but not in 8982-SF nor W9262-339A. Adeniji et al. (2010) grew isolines of W9260-BC and W9261-BG in solution with 0.1 μM $CdCl_2$; after 8 days exposure to cadmium the 'high' isolines of W9260-BC contained twice as much cadmium as did the 'low' isolines but there was no difference in cadmium content between 'high' and 'low' isolines of W9261-BG. The expected difference in cadmium accumulation between the 'high' and 'low' isolines does not, therefore, appear to be reliably obtained in solution culture. Nevertheless, a general relationship between transpiration and cadmium accumulation can be determined from our experiment, and differences in transpiration between 'high' and 'low' isolines of the two pairs that did show the expected pattern (W9260-BC and W9261-BG) might be expected to correlate with differential cadmium accumulation.

As expected, daily transpiration per jar increased with time for all isolines. As a plant grows, its surface area increases and so will the volume of water transpired. When measured on a per jar basis, there was no difference in daily transpiration or total volume of water transpired between any pair of 'high' and 'low' isolines nor between cadmium-treated and control plants (Fig. 3). However, the isolines of 8982-TL consistently transpired less water over the experimental period, which can be explained by their smaller size as compared to the other isolines. After correcting for leaf area (Fig. 4), isolines of 8982-TL and W9206-BC are seen to have transpired equal volumes of water per cm^2 of leaf tissue. Interestingly, the isolines of W9261-BG grown in the presence of cadmium transpired more per unit leaf area than did their respective controls. Barcelo and Poschenrieder (1990), in their review, provide a possible explanation: in some species, low doses of cadmium induce a higher stomatal density, inhibit stomatal closing and, if the photosynthetic rate is not affected, sugar concentrations and reduced osmotic potentials can increase in leaf tissues — all resulting in higher rates of transpiration. The reason why the isolines of W9261-BG showed this effect, when the others did not, might be related to the observation that they were the only isolines to experience a slight reduction in biomass in response to cadmium. In isolines of W9621-BG, 0.1 μM $CdCl_2$ may have been sufficient to trigger cadmium-induced changes in stomatal density or functioning but not affect photosynthetic rate.

The hypothesis that greater cadmium accumulation in shoots of the 'high' isolines would be positively correlated with volume of water transpired was not supported by our data. Indeed, the individual replicates of the 'high' isolines of W9260-BC and W9261-BG contained higher concentrations of cadmium relative to their 'low' counterparts across a range of volumes of water transpired (approx. 200–300 ml; Fig. 5b).

However, our experiment has identified an important difference between 'high' and 'low' isolines with respect to transpiration. Strong positive correlations indicate that transpiration was related to cadmium uptake from solution and translocation to the shoot in the 'low' isolines. In contrast, the uptake and translocation of cadmium to the shoots of the 'high' isolines did not vary with volume of water transpired. This means that the 'low' isolines likely retain more cadmium in the root tissues, as compared to the 'high' isoline, making proportionately less cadmium available to enter the transpiration stream. Others have also speculated that the physiological difference between 'high' and 'low' cadmium-accumulators might lie in the sequestration of cadmium in the roots. This idea has been applied to isolines of durum wheat (Adeniji et al. 2010; Harris and Taylor 2004) as well as the cultivars Kyle and Arcola (Chan and Hale 2004) and relative cadmium translocation in bread wheat (*Triticum aestivum* L.) and durum wheat (Hart et al. 1998).

From this study we can conclude that, while transpiration contributes to the total amount of cadmium in a plant, further investigations must be performed to determine the physiological difference between 'high' and 'low' accumulating isolines of durum wheat — a difference that results in relatively less cadmium entering the transpiration stream of the 'low' isolines.

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