Analysis of cadmium translocation, partitioning and tolerance in six barley (*Hordeum vulgare* L.) cultivars as a function of thiol metabolism

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Abstract

Six barley cultivars widely differing for cadmium (Cd) tolerance, partitioning and translocation were analyzed in relation to their thiol metabolism. Results indicated that Cd tolerance was not clearly related to the total amount of Cd absorbed by plants, resulting instead closely dependent on the capacity of the cultivars to trap the metal into the roots. Such behaviors suggested the existence of root mechanisms preserving shoots from Cd-induced oxidative damages, as indicated by the analysis of thiobarbituric acid-reactive-substances – diagnostic indicators of oxidative stress – whose increases in the shoots were negatively related to Cd root retention and tolerance. Cd exposure differentially affected glutathione (GSH) and phytochelatin (PC) levels in the tissues of each barley cultivar. The capacity to produce PCs appeared as a specific characteristic of each barley cultivar, since it did not depend on Cd concentration in the roots and resulted negatively related to the concentration of the metal in the shoots, indicating the existence of a cultivar-specific interference of Cd on GSH biosynthesis, as confirmed by the existence of close positive linear relationships between the effect of Cd on GSH levels and PC accumulation in both roots and shoots. The six barley cultivars also differed for their capacity to load Cd ions into the xylem, which was negatively related to PC content in the roots. Taken as a whole these data indicated that the different capacity of each cultivar to maintain GSH homeostasis under Cd stress may strongly affect PC accumulation and, thus, Cd tolerance and translocation.

Keywords: *Hordeum vulgare* L.; Cadmium; Glutathione; Thiols

Introduction

Cadmium (Cd) is one of the most toxic heavy metals present in soils from natural and anthropogenic sources, including atmospheric depositions from mining activities, phosphate fertilizers and manures, municipal sewage wastes, urban composts and industrial sludges (Alloway and Steinnes 1999; McLaughlin et al. 1999).

The presence of Cd in soils is an increasing concern with respect to human food chain accumulation, since it can be easily taken up by roots and accumulated in vegetative and reproductive plant organs: in this way, Cd-rich soils potentially result in Cd-rich foods.

Despite several efforts aimed at both reducing Cd input into agricultural soils and developing agronomic practices having the potential to reduce Cd bioavailability, breeding of low Cd-accumulating crops seems to be the most promising approach to minimize the dietary intake of Cd (Grant et al. 2008). Selection of novel cultivars with different Cd accumulation profiles should reduce not only the total amount of the heavy metal in the edible parts of the plants, but also the requirement for other management techniques. In such a context it appears evident the need to characterize and exploit the natural variation occurring in main crop species for their capacity to accumulate/exclude Cd from the edible parts, as well as to understand potential processes and molecular components that underlie these traits (Grant et al. 2008; Clemens et al. 2013).

Considerable natural variation in plant Cd accumulation occurs both between and within species (Guo et al. 1995; Grant et al. 1998; Cakmak et al. 2000; Clarke et al. 2002; Dunbar et al. 2003; Grant et al. 2008; Uraguchi et al. 2009). Most plant species retain much of the Cd taken up within roots by a conserved 'firewall system' limiting the spread of Cd through the whole plant and preventing excessive Cd accumulation into seeds (Jarvis et al. 1976; Wagner 1993; Lozano-Rodríguez et al. 1997; Puig and Peñarrubia 2009; Verbruggen et al. 2009; Ueno et al. 2010; Nocito et al. 2011). The efficiency of this system is thought to be pivotal in determining the "Cd accumulation profiles" observed in crop species.

Once inside root cells Cd ions are trapped into roots through selective binding sites with high affinity for the metal, or through transfer across a membrane into an intracellular compartment (Clemens 2006; Ueno et al. 2010; Nocito et al. 2011). Only Cd ions escaping these trapping pathways may be potentially available to be loaded, by specific transport systems, into the xylem and translocated in a root-to-shoot direction. Thus, the ability of the root system to retain Cd should result from a complex equilibrium between different biochemical and physiological processes involved in Cd chelation, compartmentalization, adsorption and translocation (Nocito et al. 2011). Several actors have been described as active members of this firewall system, including: i) the processes of Cd chelation and vacuolar compartmentalization based on the biosynthesis of phytochelatins (PCs) and

related peptides (Cobbet 2000; Clemens 2006); ii) the adsorption of Cd ions to cellular matrices or apoplast components (Weigel and Jäger 1980; Khan et al. 1984); iii) the transport-mediated sequestration of Cd ions into the vacuole (Ueno et al. 2010; Satoh-Nagasawa et al. 2013); iv) the P_{1B}-type ATPase-mediated Cd loading into the xylem (Nocito et al. 2011; Satoh-Nagasawa et al. 2012, 2013; Mills et al. 2012; Takahashi et al. 2012; Tan et al. 2013).

Recent progress in understanding the molecular mechanisms controlling Cd allocation in rice makes realistic the development of low Cd-accumulating cultivars in an immediate future (Uraguchi and Fujiwara 2012; Clemens et al. 2013). Unfortunately, not nearly as much information is available for other major cereals, including barley, for which a significant increase in grain and flour consumption is expected in some critical arid and semiarid regions of North Africa (Bei et al. 2012). Although some report about genotypic diversity in barley grain Cd accumulation exists (Wu et al. 2003, 2007; Chen et al. 2008), scarce information about the physiological basis governing Cd distribution in the plant is available. Recently, it has been shown that the preferential retention of Cd in root of barley is mainly due to immobilization processes mediated by S-ligands and reflects the accumulation of Cd-PC and Cd-S molecules in the vacuoles (Akhter et al. 2013).

In this paper we describe and compare six barley cultivars differing for their capacity to accumulate Cd in the shoot, with the specific aim to describe the role of thiol biosynthesis and metabolism in determining Cd partitioning and tolerance.

Material and Methods

Plant material, growth conditions and sampling

All the experiments were carried out on 6 varieties of barley (*Hordeum vulgare* L.) with six (Manel, Rihane, Martin, Souihli, Lemsi) or two rows (Roho) – selected among the most cultivated in Tunisia for their capacity to accumulate Cd in the shoot – provided by the National Research Agronomic Institute of Tunisia.

Surface sterilized caryopses were placed on a filter paper saturated with distilled water and incubated in the dark at 26 °C. Seven days later, seedlings were transplanted into 5 L plastic tanks (8 seedlings per tank) containing the following complete aerated nutrient solution: 1.5 mM $MgSO_4$, 1.6 mM KH_2PO_4, 0.4 mM K_2HPO_4, 3.0 mM KNO_3, 2.0 mM NH_4NO_3, 3.5 mM $Ca(NO_3)_2$, 62 µM Fe-tartrate, 9 µM $MnCl_2$, 0.3 µM $CuSO_4$, 0.8 µM $ZnSO_4$, 46 µM H_3BO_3, 0.1 µM $(NH_4)_6Mo_7O_{24}$ (pH 6.5). Seedlings were kept for 10 d in a growth chamber at 26°C and 80% relative humidity during the 16-h light period and at 22°C and 70% relative humidity during the 8-h dark period. Photosynthetic photon flux density was 400 µmol m^{-2} s^{-1}. At the end of this period, plants were treated or not (control) with Cd by supplementing the nutrient solution with $CdCl_2$ to reach the final concentration of 25 µM. The treatment period was 30 d long. All hydroponic solutions were renewed 3 times per week to minimize nutrient depletion.

Plants were harvested and roots were washed for 10 min in ice-cold 5 mM $CaCl_2$ solution to displace extracellular Cd (Rauser 1987), rinsed in distilled water and gently blotted with paper towels. Shoots were separated from roots and the tissues were frozen in liquid N_2 and stored at -80 °C, or analyzed immediately.

Determination of Cd

Dried samples of about 150 mg were digested in 10 mL of 65% (v:v) HNO_3 using a microwave digestion system (Anton Paar MULTIVAWE 3000). The mineralized material was diluted 1:40 (v:v) in Milli-Q water (to a final volume of 10 mL) and filtered on a 0.45 µm PVDF membrane. Cd content was measured by inductively coupled plasma mass spectrometry (ICP-MS; Bruker Aurora M90 ICP-MS).

Determination of thiols and thiobarbituric acid-reactive-substances

Samples (roots and shoots) were pulverized using mortar and pestle in liquid N_2 and stored frozen in a cryogenic tank. For total non-protein thiol (NPT) content, 400 mg of powders were extracted in 600 µL of 1M NaOH and 1 mg mL^{-1} $NaBH_4$, and the homogenate was centrifuged for 15 min at 13 000 *g* and 4 °C. Four hundred microliters of supernatant were collected, 66 µL of 37% HCl were added and then centrifuged again for 10 min at 13000 *g* and 4 °C. For the quantification, volumes of 200 µl of the

supernatant were collected and mixed with 800 µl of 1 M K-Pi buffer (pH 7.5) containing or not 0.6 mM Ellman's reagent {[5,5'-dithiobis(2-nitrobenzoic acid); DTNB]}. The samples' absorbances at 412 nm were then spectrophotometrically measured. The level of total GSH was determined according to Griffith (1980). Phytochelatins and related peptides were evaluated as difference between NPT and GSH levels in both root and shoot of Cd exposed plants (Schäfer et al. 1997). All results were expressed as micromoles of GSH equivalents.

The thiobarbituric acid-reactive-substances (TBARS) assay was performed according to Hodges et al. (1999).

Analysis of root-to-shoot Cd translocation

At the end of the exposure period, shoots were cut at 2 cm above the roots with a microtome blade. Xylem sap exuded from the lower cut surface was collected by trapping into a 1.5 mL plastic vial filled with a small piece of cotton for 2 h. The amount of collected sap was determined by weighing and the Cd concentration was measured by ICP-MS.

Results and discussion

Cd tolerance and partitioning in six barley cultivars

Six Tunisian improved barley cultivars – Lemsi, Manel, Martin, Rihane, Roho and Souihli – derived from local (Tunisia, Algeria) landraces (Chaabane et al. 2009), were exposed to 25 μM Cd^{2+} for 30 days and then analyzed for Cd partitioning and tolerance.

At the end of the incubation period no visible symptoms of toxicity (necrosis or chlorosis) were detectable in the shoots of any of the six barley cultivars. Such observations were confirmed by chlorophyll analysis showing that the concentration of chlorophyll a/b in the shoots was unaffected by Cd exposure (data not shown). Conversely, the growth of the six cultivars was significantly ($p < 0.001$) influenced by Cd (Fig. 1). Considering the shoots: i) Lemsi appeared to be the most sensitive cultivar, with a Tolerance Index (TI) – defined as the average weight of shoots in treated group × 100 / the average weight of shoots in control group – of 37%; ii) Roho, Martin and Souihli showed an intermediate sensitivity, with TIs of 63, 67 and 73%, respectively; iii) Manel and Rihane were the most tolerant cultivars, with TIs of 86 and 85%, respectively (Fig. 1a). Root growth was generally less affected by Cd exposure: the percentage of growth inhibition ranged from 0 in Souihli to 37% in Lemsi (Fig. 1b). Similar behaviors were evinced by referring to plant fresh weight, since Cd exposure did not affect tissue water contents (data not shown).

Wide differences were observed considering the concentration of Cd in the shoot: i) Lemsi and Manel showed the highest and the lowest values, respectively; ii) in Rihane the concentration was significantly ($p < 0.05$) higher than in Manel; iii) in Martin, Souihli and Roho the values of Cd concentration were intermediate with respect to Manel and Lemsi and significantly ($p < 0.05$) higher than in Rihane (Fig. 2a). By contrast a moderate variability was observed with regard to root Cd concentration (Fig. 2b). From these data set we calculated that: i) the total amount of Cd accumulated in the whole plant was significantly ($p < 0.05$) higher in Lemsi, Rihane, Manel, and Martin than in Roho and Souihli (Electronic Supplementary Material Tab. S1); ii) the Cd root retention (i.e. the percentage of the total Cd retained in the root) widely differed among the six cultivars (Electronic Supplementary Material Tab. S1). The lowest value of retention was observed in Lemsi (70.8%), whilst the highest one in Manel (85.9%); all the other cultivars had intermediate values.

It has been largely reported that plant responses to Cd exposure involve a plethora of constitutive and adaptive processes, which interactions at molecular, physiological and morphological level result in complex phenomena allowing the cells to protect themselves against the injury due to Cd accumulation, or allowing the plants to exclude Cd stress (Turner 1994; Gwozdz et al. 1997; Sanità di Toppi and Gabbrielli 1999; Nocito et al. 2007). Cd tolerance and Cd root-to-shoot translocation are often negatively related (Verkleij et al. 1990; Wong and Cobbett 2009). However, although tolerance

is often associated with a high capability to retain the metal into roots, it does not necessarily mean that increased root retention itself is the cause of tolerance, since intraspecific differences in Cd uptake might occur (Lombi et al. 2000; Assunção et al. 2003).

Considering our data, it is important to note that the fraction of the absorbed metal translocated to the shoot was 2.2-fold higher in Lemsi than in Manel, although they did not significantly ($p < 0.05$) differed for the total amount of Cd accumulated in the whole plant. Data analysis also revealed the lack of any clear relationship between the total amount of Cd absorbed by plant and the calculated TIs (Fig. 3a), which instead increased as Cd root retention did (Fig. 3b). Thus, at least in our conditions, the reduced capacity to absorb Cd showed by some barley cultivars - even if conceivable as a possible mechanism of stress avoidance – was not involved in Cd tolerance.

Taken as a whole this group of data suggest the existence of root mechanisms limiting Cd translocation from root to shoot and thus preserving the photosynthetic tissues from the detrimental effects that Cd may induce. In fact, although Cd is not a redox-reactive metal, its accumulation in plant tissues generally results in oxidative stress (Nocito et al. 2008; Sharma and Dietz 2009; Del Buono et al. 2014).

For this reason, to better understand the relationship between Cd root retention and Cd tolerance, we measured, at the end of the Cd exposure period, the levels of thiobarbituric acid-reactive-substances (TBARS) in the shoots, assuming these values as diagnostic indicators of the occurrence/severity of Cd-induced oxidative stress (Hodges et al. 1999). As reported in Figure 4a, Cd exposure increased the levels of TBARS in the shoots. However, such an increase strongly differed among the six barley cultivars – ranging from 171% (Manel) to 544% (Lemsi) – and resulted negatively related to Cd tolerance (Fig 4b), suggesting Cd root retention as a possible mechanism of stress avoidance which preserves shoot tissues from Cd-induced oxidative damages. Finally, the importance of such a mechanism in determining Cd tolerance is further supported by the following observations: i) TI values increased as Cd concentration in the shoot decreased (Fig 2a and Fig. 3); ii) Cd-induced oxidative damages increased as Cd concentration in the shoot did (Fig 2a and Fig. 4). In this way, the selection of novel genotypes with enhanced Cd root retention or/and lower Cd concentration in the shoot may represent a valuable strategy, not only to reduce Cd exposure through plant-derived food, but also to increase Cd tolerance.

Analyses of Cd partitioning and tolerance as a function of thiol metabolism

Plant sulfur metabolism and thiol biosynthesis are deeply affected by Cd stress, mainly because of the activation of a wide range of adaptive responses involving glutathione (GSH) consuming activities (Nocito et al. 2006, 2007; Lancilli et al. 2014). In fact, GSH not only acts as a direct or indirect antioxidant in mitigating Cd-induced oxidative stress, but also represents a key intermediate for the

316 synthesis of phytochelatins, a class of cysteine-rich peptides able to form thiolate bonds with Cd ions
317 in complexes that accumulate in the vacuoles (Cobbet 2000; Clemens 2006). Studies on maize, rice and
318 barley showed that most of the total Cd retained by roots is bound in complexes containing PCs and
319 related thiol compounds, revealing these peptides as crucial for Cd root retention in cereals (Rauser
320 and Meuwly 1995; Rauser 2003; Nocito et al. 2011; Akhter et al. 2013). Since the activity of
321 homeostatic mechanisms based on thiol biosynthesis has been shown to be involved in Cd tolerance
322 and may potentially allow a different proportion of Cd to be retained in roots, we analyzed the effects
323 of Cd exposure on GSH and non-protein thiol (NPT) levels in both roots and shoots of the six barley
324 cultivars.

325 Cadmium exposure significantly ($p < 0.001$) reduced the levels of total GSH in both roots and
326 shoots of all the cultivars (Fig. 5a,d). Such an effect was likely due to a general alteration of thiol
327 homeostasis as indicated by the analysis of the NPTs, which levels in both roots and shoots significantly
328 ($p < 0.001$) increased following Cd stress and overcame those of GSH – the main non-protein thiol in
329 non-stressed plant tissues – measured in the same conditions (Fig. 5b,e).

330 Data analysis revealed that the entity of the GSH decrement induced by Cd was negatively
331 related to the general tolerance of the six barley cultivars to Cd stress. In fact, the effect of Cd on GSH
332 content was minimum (or absent) in Manel and maximum in Lemsi, considering both roots and shoots
333 (Electronic Supplementary Material Fig. S1 a,b). Conversely, the increments in the NPT content induced
334 by Cd were directly related to the Cd tolerance: the highest increase was observed in Manel (+359%),
335 whilst the lowest one was measured in Lemsi (+10%; Electronic Supplementary Material Fig. S1 c,d).
336 PC and related peptide contents (Fig. 5c,f) were evaluated as difference between NPT and GSH levels
337 in both roots and shoots of Cd-exposed plants (Schäfer et al. 1997). Results indicated that the six barley
338 cultivars widely differed for their capacity to synthetize PCs and related peptides (Fig. 5c,f). Also in this
339 case the level of these compounds in both roots and shoots was closely related to the Cd tolerance of
340 each cultivar (Electronic Supplementary Material Fig. S1 e,f).

341 Cd exposure rapidly induces PC biosynthesis in plant tissues as result of GSH polymerization
342 through the constitutive enzyme phytochelatin synthase (Rea et al. 2004). Short-term exposures to Cd
343 generally result in both PC accumulations and GSH depletions closely related to the total amount of
344 the metal accumulated in the tissues. In such a context the decreases in GSH levels due to the induction
345 of PC biosynthesis should be directly related to the amount of PCs accumulated in the tissues or, in
346 other words, to the strength of the additional sinks for reduced sulfur induced by Cd (Grill et al. 1987;
347 Tukendorf and Rauser 1990; Mendoza-Cózatl and Moreno-Sánchez 2006). However, under long-term
348 Cd exposures PCs rapidly become the most abundant class of non-protein thiols and the relative
349 increase in the metabolic demand for both cysteine and GSH generates a typical demand driven
350 coordinated transcriptional regulation of genes involved in sulfate uptake, sulfate assimilation and GSH

biosynthesis (Nocito et al. 2007). Such a response is thought to be pivotal in a metabolic scenario in which the rate of GSH biosynthesis has to maintain not only GSH homeostasis but also PC-based Cd detoxification processes (Nocito et al. 2007).

The analysis of thiols revealed the existence of a general relationship between the capacity of the barley cultivars to synthetize PCs and their Cd tolerance (Electronic Supplementary Material Fig. S1 e,f), which however did not seem related to the total amount of Cd accumulated (Fig. 3a), as previously reported by Persson et al. (2006). The capacity to produce and accumulate PCs appeared as a specific characteristic of each barley cultivar since it was not significantly related to Cd concentration in the roots and resulted negatively related to the quantity of Cd accumulated in the shoot (Electronic Supplementary Material Fig. S1 g,h). Moreover, considering GSH concentrations in both root and shoot of untreated plants (control) it appears evident the lack of any clear relationship between the total amount of reduced sulfur assimilated into GSH and the tolerance of each cultivar to Cd stress. These behaviors may reflect any difficulties in maintaining GSH homeostasis during Cd stress and could be ascribed to a direct and cultivar-specific interference of Cd on some activity along the pathways involved in sulfate uptake, sulfate assimilation and GSH biosynthesis.

Such a hypothesis seemed to be confirmed by the analyses of the changes in the GSH levels induced by Cd accumulation which showed the existence of close positive linear relationships between the effect of Cd on GSH levels and PC accumulation in both root and shoot (Fig. 6a,b). In other words the ability of each barley cultivars to maintain GSH homeostasis during PC biosynthesis was crucial for Cd tolerance, as previously demonstrated by the analysis of transgenic *Brassica juncea* plants in which the over-expression of γ-glutamylcysteine synthetase or GSH synthetase – the two enzymes along the GSH biosynthetic pathway – enhanced Cd tolerance as a consequence of a greater production of GSH during Cd stress (Zhu et al. 1999a, 1999b). On the other hand, transgenic Arabidopsis plants expressing the cDNA for γ-glutamylcysteine synthetase in antisense orientation resulted hypersensitive to Cd as a consequence of a reduced capacity to synthetize both GSH and PCs under the exposure to the metal (Xiang et al. 2001).

Analysis of root-to-shoot Cd translocation as a function of thiol metabolism

To better understand the relationship existing between Cd root retention, thiol biosynthesis and root-to-shoot Cd translocation we measured the concentration of Cd in the xylem sap of the six barley cultivars at the end of the exposure period. In these experiments Cd translocation was estimated as the amount of Cd ions loaded and transported in the xylem sap for 2 h, according to Nocito et al. (2011).

Results indicated that the six barley cultivars strongly differed for their capacity to load Cd ions into the xylem (Fig. 7a). The amount of Cd transported in the xylem sap of the six barley cultivars during

386 the observation period ranged from 55.3 (Manel) to 187.5 ng 2 h^{-1} (Lemsi), and was linearly related (r^2
387 = 0.817) to the total amount of Cd accumulated in the shoots over a 30 d period (Fig. 7b).
388 Since the capacity of barley roots to retain Cd ions has been recently associated to
389 immobilization processes mediated by S-ligands (Akhter et al. 2013), we analyzed Cd translocation as
390 a function of GSH homeostasis and PC accumulation in the roots, with the aim to evince a general
391 relationship describing how the "Cd translocation" trait depends on root thiol metabolism in different
392 barley genotypes. Results revealed that Cd translocation was closely related to thiols since the amount
393 of Cd ions loaded in the xylem sap linearly decreased as PC content in the roots increased (Fig. 7c).
394 Moreover, since the capacity of the roots to synthetize PCs was related to the capacity of each cultivar
395 to maintain GSH homeostasis, it was also possible to evince a negative relation between Cd
396 translocation and the negative effect exerted by Cd on GSH biosynthesis (Fig. 7d). Such an analysis
397 allows us to speculate that the genotypic differences observed in Cd translocation in the six barley
398 cultivars could be partially due to a different sensitivity of GSH metabolism to Cd accumulation. In this
399 view the different capacity of each barley cultivar to maintain GSH homeostasis during Cd stress should
400 affect PC production and, thus, Cd translocation capacity, since, in the absence of any other significant
401 differences in the main components of the firewall trapping Cd into the roots, the amount of Cd ions
402 escaping thiol chelation may be considered as potentially available to be loaded into the xylem and
403 translocated in a root-to-shoot direction.
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405 *Conclusions*
406 Taken as a whole our analysis confirms the central role of both GSH and PCs in determining Cd
407 tolerance and partitioning, and suggests that the effect of Cd on GSH biosynthesis may be potentially
408 taken into account to develop indexes useful for the selection of low Cd-accumulating cultivars in
409 barley. However, the molecular bases of such an effect need to be further investigated in order to
410 individuate the main factor(s) – along the sulfur metabolic pathways – influencing the capacity of
411 barley to maintain GSH homeostasis during Cd-induced PC biosynthesis. Interestingly, Schneider and
412 Bergmann (1995) indicated the activity GSH synthetase as a possible limiting factor. Finally, our
413 conclusions need to be validated in open field or glasshouse experiments, in where the activity of root
414 exudation (Cesco et al. 2012) and the presence of rhizobacteria (Palacios et al. 2014) may also influence
415 plant Cd uptake and tolerance.
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Figure legends

Fig. 1 Effect of Cd exposure on growth of shoots (a) and roots (b) of six barley cultivars. Plants were grown for 30 days in a complete nutrient solution supplemented (black bars) or not (white bars) with 25 µM $CdCl_2$. Bars and error bars are means and SD of three experiments each performed with 4 plants (n = 3). Asterisks indicate significant differences between control and Cd-exposed plants ($p < 0.001$). Different letters indicate significant differences between the cultivars ($p < 0.05$).

Fig. 2 Cadmium accumulation in shoots (a) and roots (b) of six barley cultivars. Plants were grown for 30 days in a complete nutrient solution supplemented with 25 µM $CdCl_2$. Bars and error bars are means and SD of three experiments each performed with 4 plants (n = 3). Different letters indicate significant differences between the cultivars ($p < 0.05$).

Fig. 3 Analysis of Cd tolerance as a function of the total amount of Cd absorbed by plants (a) or Cd root retention (b) in six barley cultivars. Plants were grown for 30 days in a complete nutrient solution supplemented or not with 25 µM $CdCl_2$. Data are means and SD of three experiments each performed with 4 plants (n = 3). TI, tolerance index.

Fig. 4 Effect of Cd exposure on the levels of TBARS in the shoots of six barley cultivars (a) and analysis of Cd tolerance as a function of changes in TBARS content (b). Plants were grown for 30 days in a complete nutrient solution supplemented (black bars) or not (white bars) with 25 µM $CdCl_2$. Data are means and SD of three experiments each performed with 4 plants (n = 3). TI, tolerance index. Asterisks indicate significant differences between control and Cd-exposed plants ($p < 0.001$). Different letters indicate significant differences between the cultivars ($p < 0.05$).

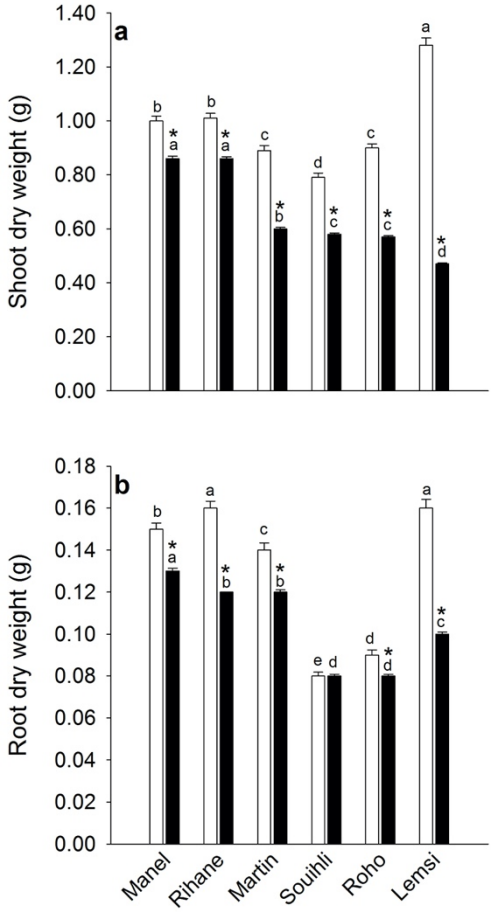
Fig. 5 Effect of Cd exposure on the level of thiols in roots (a, b, c) and shoot (d, e, f) of six barley cultivars. Plants were grown for 30 days in a complete nutrient solution supplemented (black bars) or not (white bars) with 25 µM $CdCl_2$. NPT contents are expressed as GSH equivalents. PCs were evaluated as difference between NPT and GSH levels in both roots and shoots of Cd-exposed plants. Bars and error bars are means and SD of three experiments each performed with 4 plants (n = 3). Asterisks indicate significant differences between control and Cd-exposed plants ($p < 0.001$). Different letters indicate significant differences between the cultivars ($p < 0.05$).

Fig. 6 Analysis of PC content as a function of the effect of Cd on GSH levels in roots (a) and shoots (b) of six barley cultivars. Plants were grown for 30 days in a complete nutrient solution supplemented or

not with 25 µM $CdCl_2$. Changes in GSH content were calculated comparing the GSH contents both roots and shoots of control and Cd-exposed plants. PCs were evaluated as difference between NPT and GSH levels in both roots and shoots of Cd-exposed plants. Data are means and SD of three experiments each performed with 4 plants (*n* = 3).

Fig. 7 Analysis of Cd translocation in six barley cultivars. Plants were grown for 30 days in a complete nutrient solution supplemented or not with 25 µM $CdCl_2$. At the end of the exposure period, shoots were separated from roots and the xylem sap exuded from the cut (root side) surface was collected. (a) Cd ions loaded and transported in the xylem sap during 2 h. Data are means and SD of three experiments each performed with 4 plants (*n* = 3). Different letters indicate significant differences between the cultivars ($p < 0.05$). (b, c, d) Relationships between Cd ions loaded in the xylem sap, Cd concentration in shoots, and changes in root thiol content after a 30 d period of Cd exposure. Data are means and SD three experiments each performed with 4 plants (*n* = 3).

Figure 1


a
Shoot dry weight (g)
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
b
Root dry weight (g)
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Manel
Rihane
Martin
Souihli
Roho
Lemsi

682 Figure 2

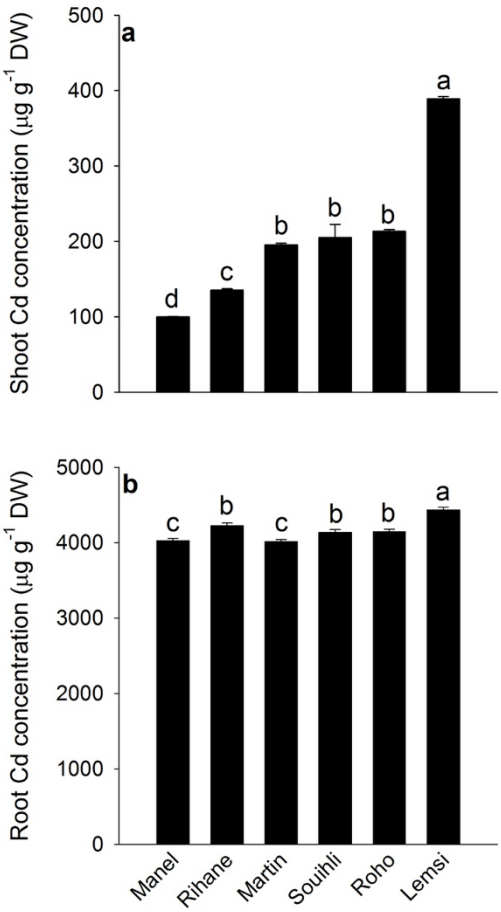


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Figure 3

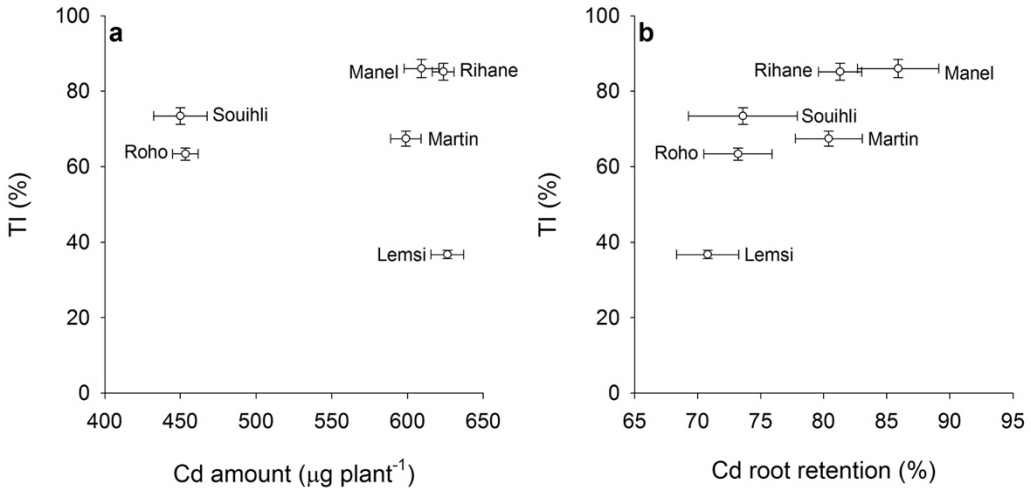

a
b
TI (%)
Cd amount (μg plant-1)
Cd root retention (%)
Manel
Rihane
Souihli
Martin
Roho
Lemsi
0
20
40
60
80
100
400
450
500
550
600
650
65
70
75
80
85
90
95

Figure 4

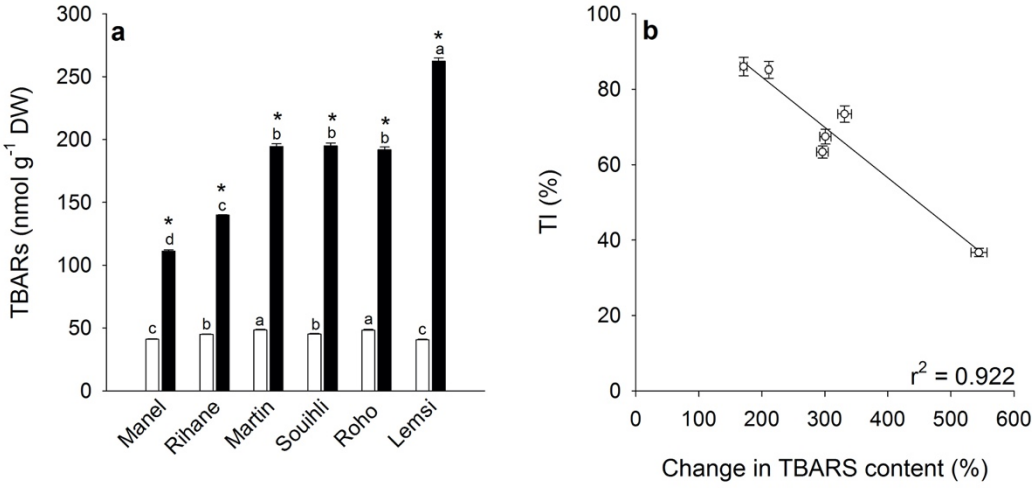

a
TBARs (nmol g-1 DW)
300
250
200
150
100
50
0
c
d
b
c
a
b
b
b
a
b
c
a
*
Manel
Rihane
Martin
Souihli
Roho
Lemsi
b
TI (%)
100
80
60
40
20
0
r2 = 0.922
0
100
200
300
400
500
600
Change in TBARS content (%)

Figure 5

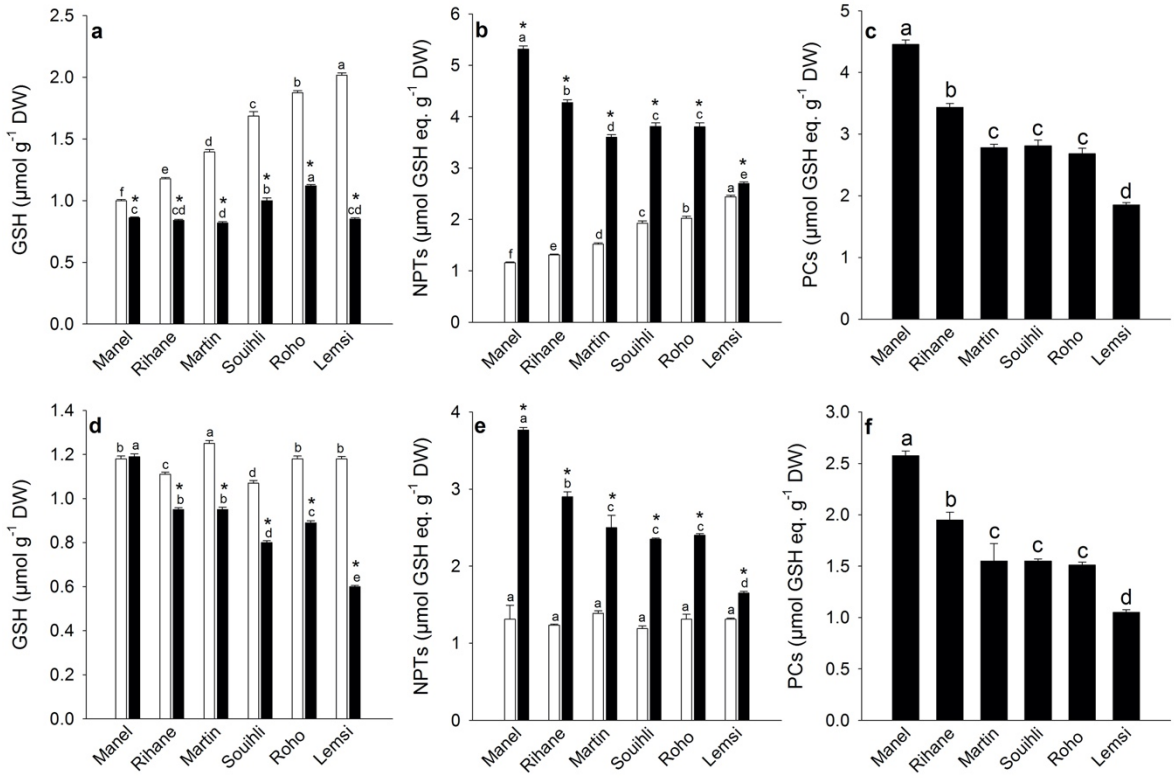
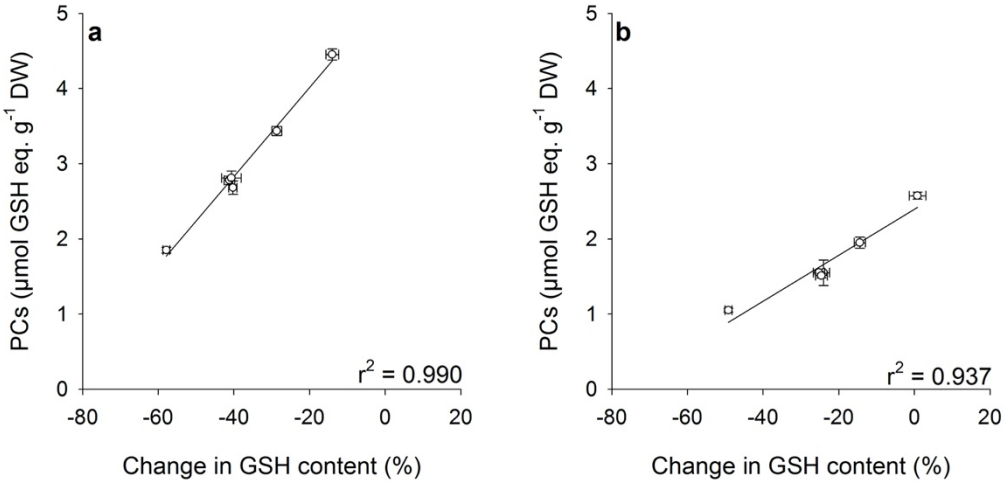
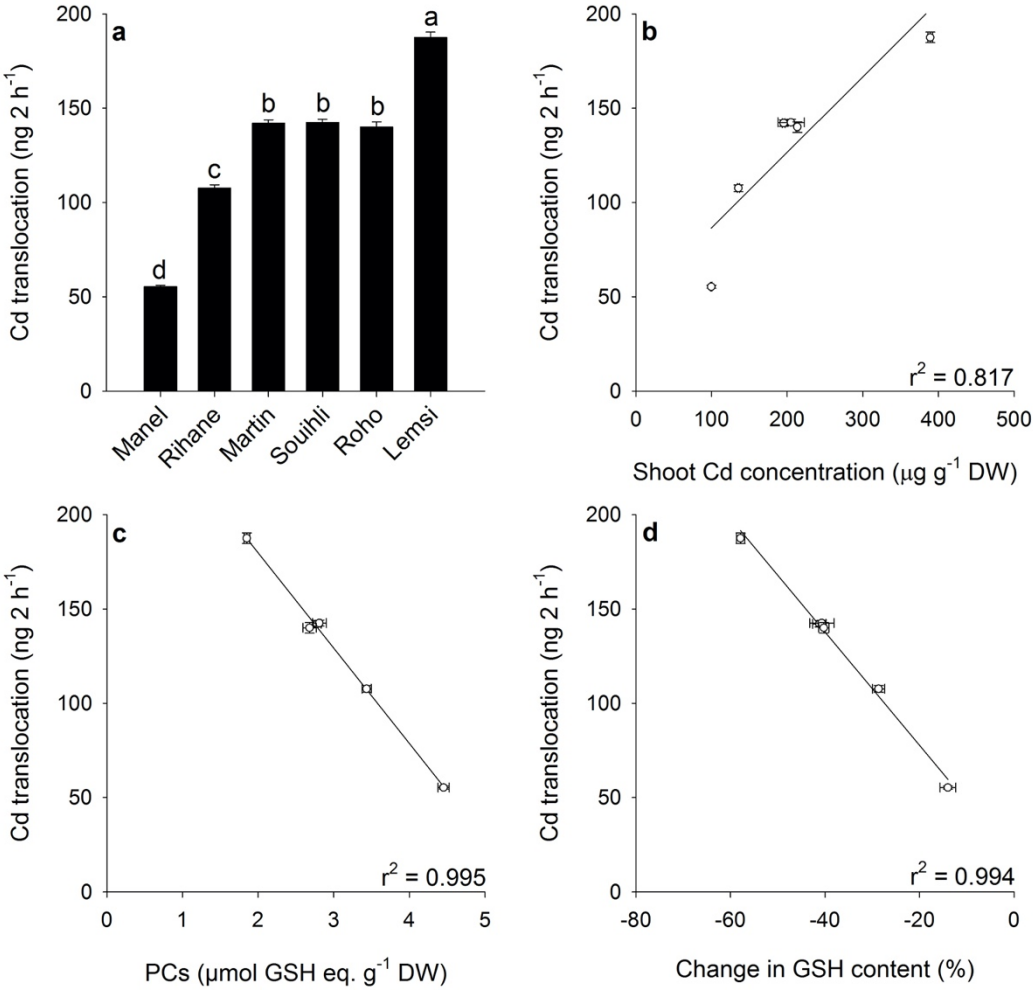

a
b
c
d
e
f
GSH (µmol g-1 DW)
NPTs (µmol GSH eq. g-1 DW)
PCs (µmol GSH eq. g-1 DW)
Manel
Rihane
Martin
Souihli
Roho
Lemsi

Figure 6


a
b
PCs (µmol GSH eq. g-1 DW)
Change in GSH content (%)
r2 = 0.990
r2 = 0.937
0
1
2
3
4
5
-80
-60
-40
-20
0
20

792 Figure 7



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