Original Research

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Journal of Environmental Biology

DOI : http://doi.org/10.22438/jeb/39/6/MRN-737

JEB

p-ISSN: 0254-8704
e-ISSN: 2394-0379
CODEN: JEBIDP

Tolerance capacity of Turkish genotypes of barley (*Hordeum vulagare* L.) for cadmium stress

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Key words

Cadmium stress
Genotype screening
Hordeum vulgare
Seed germination
Seedling growth

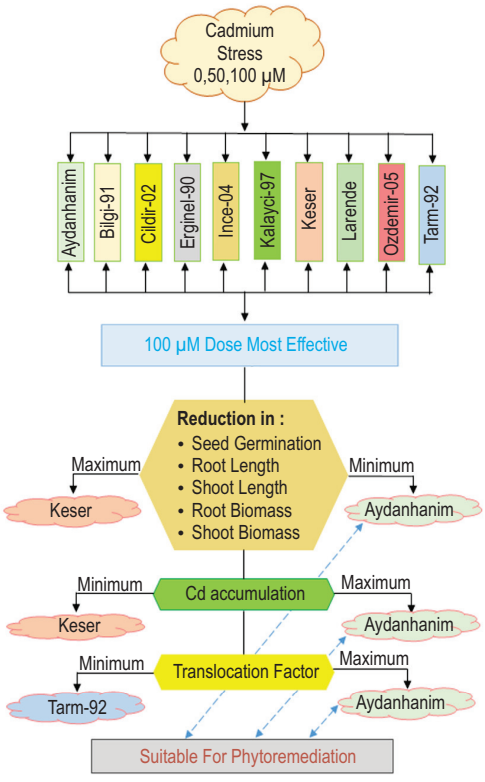
Publication Info

Paper received : 30.08.2017
Revised received : 16.12.2017
Accepted : 19.02.2018

Abstract

Aim : Elevated levels of heavy metals in agricultural soils result in significant contamination of food in Turkey, where cadmium toxicity is currently one of the most serious environmental issues. This study was undertaken to screen the various Turkish genotypes of barley for their sensitivity/tolerance towards cadmium stress.

Methodology : Short-term experiments involving plant exposure to different Cd concentrations (0–100 µM) were conducted to evaluate the response of ten barley genotypes (*viz.*, Aydanhanım, Bilgi-91, Çildir-02, Erginel-90, İnce-04, Kalayci-97, Keser, Larende, Özdemir-05, Tarm-92) under hydroponic conditions. Seed germination, seedling growth and Cd accumulation in plant tissues were taken as the criteria to evaluate the comparative genotypic response to Cd stress.



Results : Cd-induced stress significantly decreased seed germination percentage in all genotypes and the effect was dose-dependent. At 100 µM Cd stress, genotypes Keser and Aydanhanim showed the maximum (46%) and minimum (12%) loss respectively, compared with the control. Root length was reduced by 68% and 30%, while shoot length declined by 44% and 11% in these genotypes, respectively. Similarly, the loss of root and shoot biomass was maximum in genotype Keser and minimum in Aydanhanim. However, genotype Aydanhanim accumulated the maximum (5.96 µg g^{-1} d.wt.), whereas Keser retained the minimum (5.41 µg g^{-1} d.wt.) amount of Cd in their roots at 100 µM. Cd accumulation in shoot also displayed a similar genotypic difference.

Interpretation : Based on the above observations, Aydanhanım was Cd-tolerant, while Keser was most susceptible among the genotypes studied. Thus, genotype Aydanhanim has a promise to be a suitable candidate for phytoremediation of Cd-contaminated sites.

Introduction

Unplanned urbanization and rapidly expanding industrialization in developing countries have resulted in huge heavy metal discharges into the environment over the last few decades, thus posing a potential threat to ecosystems and human health (Mahmood *et al.,* 2012; Wang *et al.,* 2017). Heavy metals present in soils and/or waters are not degraded by biological or chemical processes, hence persist for long periods of time and are often taken up by plants grown in that environment (Iqbal *et al.,* 2015). The surface and ground waters, containing elevated levels of heavy metal are sometimes used as a source of drinking water and pose threat to fresh water ecosystems and human populations (Akbulut and Tuncer, 2011).

Cadmium, has contaminated the agricultural soil and become a critical environmental concern due to its potential adverse ecological effects. The regulatory limit of Cd for agricultural soils is 100 mg kg^{-1} soil (Salt *et al.,* 1995), although this threshold has been exceeded due to a variety of anthropogenic activities (Kalai *et al.,* 2014). Cd is a non-essential element for crop plants, but is readily taken up by the plants growing on Cd-contaminated soils and thus, enters the food chain (Fischer, 2005; Long *et al.,* 2013). Elevated Cd concentrations cause a number of quantifiable toxic symptoms in plants, *e.g.* they inhibit seed germination and seedling growth (Jaouani *et al.,* 2016), photosynthetic process, chlorophyll biosynthesis and nitrate reductase activity (Ali *et al.,* 2000, 2001; Mobin, 2013; Bashir *et al.,* 2015), and can alter even the morphological and anatomical characteristics of plants (Mehindirata *et al.,* 1999, 2000; Khudsar *et al.,* 2000, 2001; Ahmad *et al.,* 2005). This leads to a decline in crop productivity (Vassilev *et al.,* 1998; Anjum *et al.,* 2014) and eventually to the death of the plant (Nagajyoti *et al.,* 2010). Cadmium accumulation alters the mineral-nutrients uptake, inhibits the stomatal opening by interacting with water balance of the plant, and disturbs the Calvin cycle enzymes, carbohydrate metabolism and antioxidant system (Nazar *et al.,* 2012; Anjum *et al.,* 2008a, 2011). Crop tolerance to Cd toxicity depends on the crop's ability to absorb Cd ions. Cadmium interacts with the available soil nutrients some of which also have a protective role against the toxic effects of Cd stress (Ali *et al.,* 1998a, 1998b; Nazar *et al.,* 2012). For instance, the relationship between Cd and Zn in plants with reference to their uptake, translocation and remobilization is very complex. They have similar ionic structures and electro-negativities, but different ionic radii (Zn^{2+} = 0.074 nm, Cd^{2+} = 0.097 nm), which might correlate to plant selectivity (El-Kafafi and Rizk, 2013). The reduction in Cd uptake caused by Zn fertilization might be due to the competitive transport and absorption interaction between these two ions.

Barley (*Hordeum valgare* L.), a staple food in many parts of the world and the fourth largest cereal crop cultivated and consumed worldwide, is an ideal model crop for hereditary and physiological studies (Forster *et al.,* 2000). Owing to a rapid loss of genetic difference through genotype replacement, barley genotypes have become more sensitive to environmental stress (Ahmed *et al.,* 2013). Identification of such native barley plants that possess Cd tolerance and accumulation traits should help in the selection, breeding or genetic engineering of the future crops that are capable of growing in the contaminated environments and remediating the Cd-contaminated soils with lesser risk of food-chain contamination. It is, therefore, important to know the level of resistance to Cd toxicity in various genotypes and their capacity to accumulate Cd.

Most of the methods available for screening the heavy-metal-tolerant plants are expensive and time-consuming. Screening of a large number of genotypes in the field often suffers from spatial heterogeneity of chemical and physical properties of the soil and from seasonal fluctuations in rainfall, humidity and temperature (Mahmood, 2009). For mass screening, hydroponics-based nutrient-solution culture is an efficient and cost-effective technique, as it provides an easy access to plant-root system and a better control of nutrient management with a uniform distribution of the stressor in the nutrient media (Ansari *et al.,* 2015), although, on the other hand, the sand-culture method may predict field results more accurately.

The present study, carried out through a hydroponic culture, is a maiden attempt to screen the various Turkish genotypes of barley for their sensitivity towards Cd stress. It is focused on seed germination, seedling growth and Cd accumulation in ten barley genotypes with an aim to determine their tolerance capacity for the cadmium stress.

Materials and Methods

Seed germination and seedling growth : Seeds of ten genotypes of barley (*Hordeum vulgare* L.), viz., Aydanhanım, Bilgi-91, Çildir-02, Erginel-90, İnce-04, Kalayci-97, Keser, Larende, Özdemir-05 and Tarm-92, were procured from the Transitional Zone Agricultural Research Institute, Eskisehir, Turkey, and germinated hydroponically on Whatman filter papers (#42) soaked with 3 ml distilled water and with 3 ml of aqueous solutions of 50 and 100 µM of Cd (as $CdCl_2$) and placed in separate Petri dishes (diameter 100 mm, height 25 mm) kept in the dark for 3 days in a growth chamber maintained at 25°C. Fifteen seeds were placed in each Petri dish and each treatment was replicated thrice. The seeds were considered germinated when both plumule and radicle were extended from their junction. Seeds were counted after 10 days for calculating the germination percentage.

In another set of experiment, small seedlings were transferred, three days after germination, to plastic beakers containing 250 ml of nutrient solution, which consisted of 3 mM KNO_3, 2 mM $Ca(NO_3)_2$, 1mM $NH_4H_3PO_4$, 50 µM KCl, 25 µM H_3BO_3, 2 µM $MnCl_2$, 2 µM $ZnCl_2$, 0.5 µM $CuCl_2$, 0.5 µM $(NH_4)_6MO_7O_{24}$, 20 µM Na_2Fe-EDTA and 1mM $MgSO_4$. Concentration of Mg^{2+} was maintained at 1mM by subsequent addition of $MgCl_2$ and pH of the solution was adjusted to 6.5 ± 0.1 with 0.1 M NaOH. The nutrient solution was aerated continuously with sterile air and

replaced weekly. After 10 days growth, 0, 50 and 100 µM Cd (as $CdCl_2$) solutions were added to the nutrient solution. One set of seedlings (with 0 µM added Cd) served as the control. All the seedlings were exposed to a photosynthetic photon flux density of 200 µmol m^{-2} s^{-1} using a combination of fluorescent tubes and tungsten lamps under a 16 hr photoperiod.

The seedlings were harvested 10 days after the application of Cd and washed thoroughly with sterilized distilled water before further analysis. The length of shoots and roots was measured using a 30 cm ruler. The shoots and roots were separated and oven-dried at 70°C for 72 hrs and then weighed. The dried biomass was subsequently ground with a stainless steel grinder and passed through a 100-mesh sieve prior to estimating the Cd content in plant parts.

Analysis of cadmium : Cadmium content in roots and shoots was determined following the digestion of dried plant material. The harvested plant parts were washed thoroughly with Milli-Q water (resistivity >18 MΩ. cm at 25°C), dried at 70 ± 2°C for 72 hrs, ground to a fine powder and digested in a sulfuric acid/nitric acid mixture (2:1, v/v), following the method of Piper (1942). Dried material (0.25 g) from each treatment was gently swirled with concentrated HNO_3 (3 ml) in a 50 ml digestion tube on a heating block maintained at 150°C for one hr. Digestion tubes were then cooled to room temperature and 30% H_2O_2 (2 ml) was added. Heating was continued for an additional 3 hrs at 150°C and thereafter, cooled to room temperature. The digested plant solution was diluted to 50 ml and the supernatant was analyzed for Cd content, using an atomic absorption spectrometer (ZEEnit 65, Analytik Jena, Germany) fitted with a Wall type graphite tube.

Statistical analysis : All the values were the means (n=9) of three independent runs of the experiments with three replications in each run. A two-way ANOVA test was used to confirm the significance of the data. Comparison with the control and among treatments was done using the Duncan's multiple range test. To determine whether differences between treatments were significant relative to the control, least significant difference (LSD, p≤05) was determined.

Results and Discussion

Seed germination, i.e., emergence of radicle from testa as visible to eye, was recorded and expressed as percentage, with the control having 100% germination. Cd stress inhibited seed germination in all the genotypes screened. The maximum effect appeared under the influence of 100 µM, wherein 13 seeds (out of 15 seeds tested) germinated in the case of genotype Aydanhanim and only 11 in the case of Keser. The decline in seed germination with reference to the control was significant and dose-dependent and varied in different genotypes from 12% (Aydanhanim) to 46% (Keser) (Fig. 1).

Plant sensitivity to toxic metals is influenced not only by the concentration and type of the toxicant, but also by the developmental stage of the plant (Kalai *et al.,* 2014). Seed germination, one of the most sensitive physiological stages in

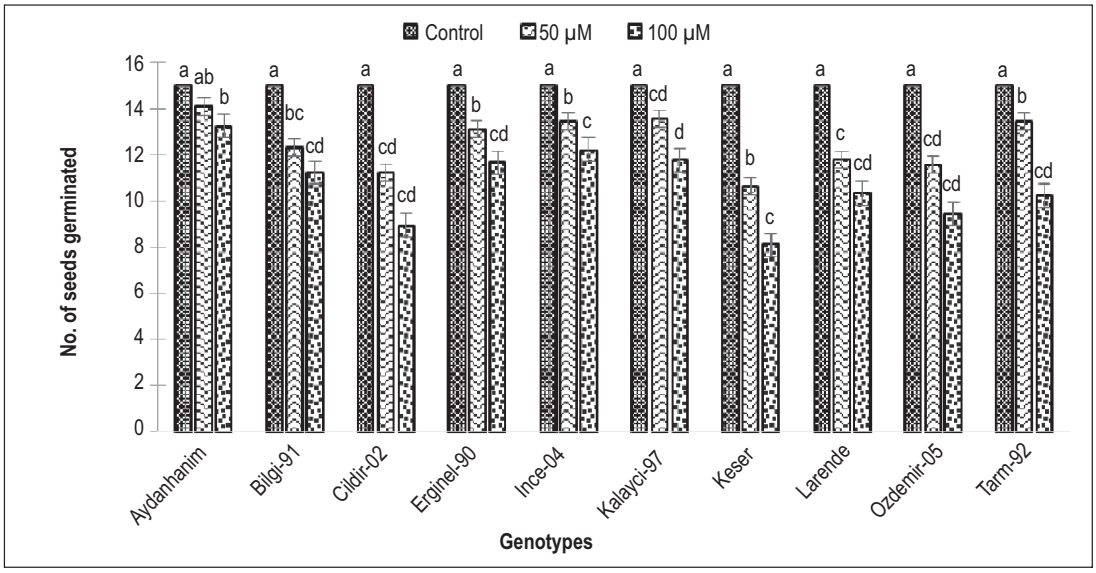


Fig. 1 : Seed germination in different barley genotypes growing under Cd stress (0–100 µM). All values are means of three independent experiments with three replicates (n=9). Vertical lines indicate ± SE. The bars bearing different letters are statistically different at p ≤ 0.5, as determined by the Duncan's Multiple Range Test.

plant growth, starts with water absorption by mature seeds and ends with the protraction of embryonic axis and the appearance of radicle through seed coats (Rajjou *et al.,* 2012). At the cellular level, seed germination is basically characterized by the resumption of respiratory activity through reactivation of glycolysis, Krebs cycle and respiratory chain (Müntz *et al.,* 2001); reserves mobilization via secretion of hydrolytic enzymes (Sfaxi-Bousbih *et al.,* 2010), de-polymerization of reserves, and transport of metabolites released into the growing embryonic cells (Smiri *et al.,* 2009); and reduction in the strength of tissues

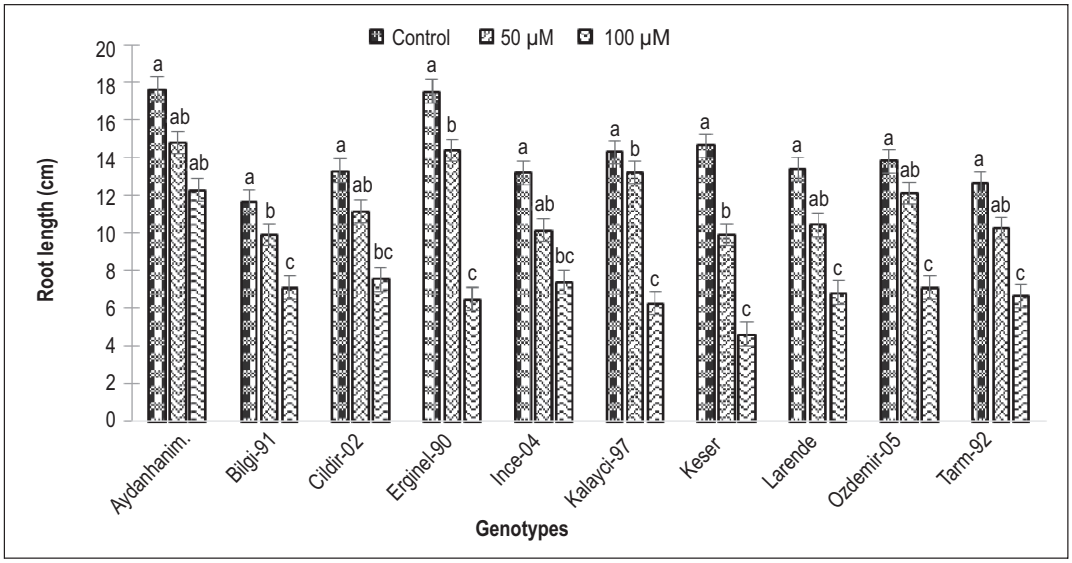


Fig. 2 : Root length of 20-day-old seedlings of barley genotypes growing under Cd stress (0–100 µM). All values are means of three independent experiments with three replications each (n=9). Vertical lines indicate ± SE. The bars bearing different letters are statistically different at p≤ 0.5, as determined by the Duncan's Multiple Range Test.

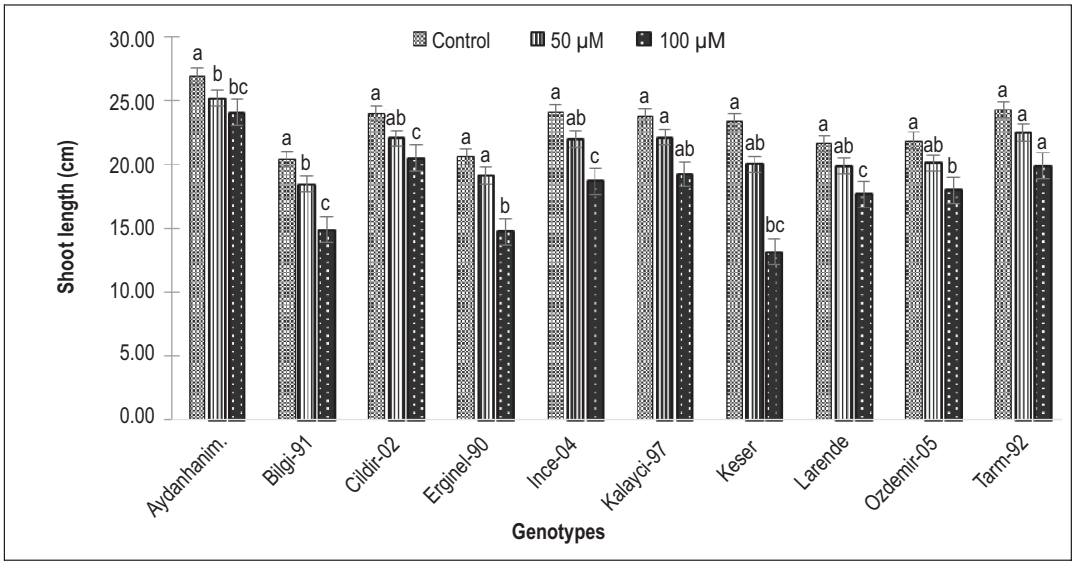


Fig. 3 : Shoot length of 20-day-old seedlings of barley genotypes growing under Cd stress (0–100 µM). All values are means of three independent experiments with three replications each (n=9). Vertical lines indicate ± SE. The bars bearing different letters are statistically different at p≤ 0.5, as determined by the Duncan's Multiple Range Test.

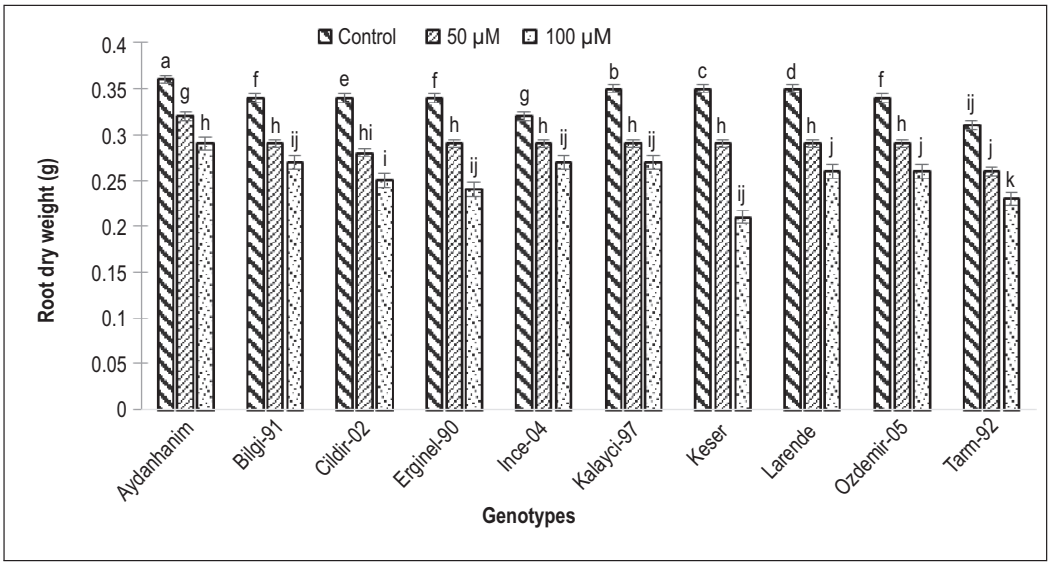


Fig. 4 : Root dry weight of 20-day-old seedlings of barley genotypes growing under Cd stress (0–100 µM). All values are means of three independent experiments with three replications each (n=9). Vertical lines indicate ± SE. The bars bearing different letters are statistically different at p≤ 0.5, as determined by the Duncan's Multiple Range Test.

surrounding the embryo, mainly due to increased activity of cell wall hydrolases (Kalai *et al.,* 2014). Moreover, seed germination relies almost exclusively on seed reserves for the supply of metabolites for respiration as well as other anabolic reactions. The available evidences indicates that starch, the most abundant storage material in seeds, is degraded in germinating seeds predominantly via the amylolytic pathway (Dua and Sawhney, 1991; Ansari *et al.,* 2013). Seed coat provides some protection from the metal stress prior to germination, but eventually cracks or becomes more permeable upon germination. Inhibition of seed germination depends, however, on type and concentration of the metal used, duration of seed exposure, plant species and grain integuments (Munzuroglu and Geckil, 2002). In germinating pea seeds, the cadmium-induced oxidative stress was resisted initially by embryos for few days, beyond which severe metabolic disturbances started, followed by protein damage, structural disruption of membranes and a disturbed cellular homeostasis, possibly due to failure of antioxidant defence system; these developments inhibited the embryonic growth (Jaouani *et al.,* 2016). Cadmium also inhibits the activity of enzymes such as alcohol dehydrogenase, hexokinase and glucose-6-phosphate dehydrogenase, as observed in germinated seeds of *Pisum sativum* (Munzuroglu and Zengin, 2006).

Metals affect seed germination by inhibiting the water uptake and disturbing the various nutritional mechanisms. Although seeds are well-protected against stresses, soon after imbibition and protrusion of embryonic axis, they become stress sensitive (Kuriakose and Prasad, 2008). In this study, seed germination was most efficient in genotype Aydanhanim and poorest in genotype Keser because the seed coats degraded quicker in the latter genotype than in the former. In short, the greater inhibition of seed germination in Keser than in Aydanhanim could be due to the suppression of water uptake or an earlier cracking of seed coat, thus causing a differential genotypic resistance to Cd toxicity.

Plant-growth inhibition due to Cd stress was observed, in this study, with a considerable decline in root length, shoot length and biomass accumulation in a dose-dependent manner maximum occurring at 100 µM dose. The root length in genotype Aydanhanim measured about 17 cm in the control population and 12 cm under 100 µM Cd stress, while the Keser roots measured about 11 cm and 5 cm at 0 µM and 100 µM, respectively (Fig. 2), showing that the reduction in the root length caused by 100 µM Cd varied between 30% (Aydanhanim) and 68% (Keser) with respect to the control. Likewise, the shoot length was nearly 27cm and 24 cm in genotypes Aydanhanim, whereas 23 cm and 13 cm in genotype Keser under 0 µM and 100 µM Cd stress respectively (Fig. 3), showing an average reduction of 11% (Aydanhanim) and 44% (Keser) with 100 µM Cd stress.

Since growth is irreversible, the increase in the mass, weight or volume of a living system, the size and biomass of plant parts are considered as indices of growth performance. Cadmium is known to exert adverse effects on plant growth, especially at early developmental stages (Anjum *et al.,* 2008b; Song *et al.,* 2014), causing growth arrest, wilting, leaf necrosis and root browning (Song *et al.,* 2014). Anatomical and physiological disturbances

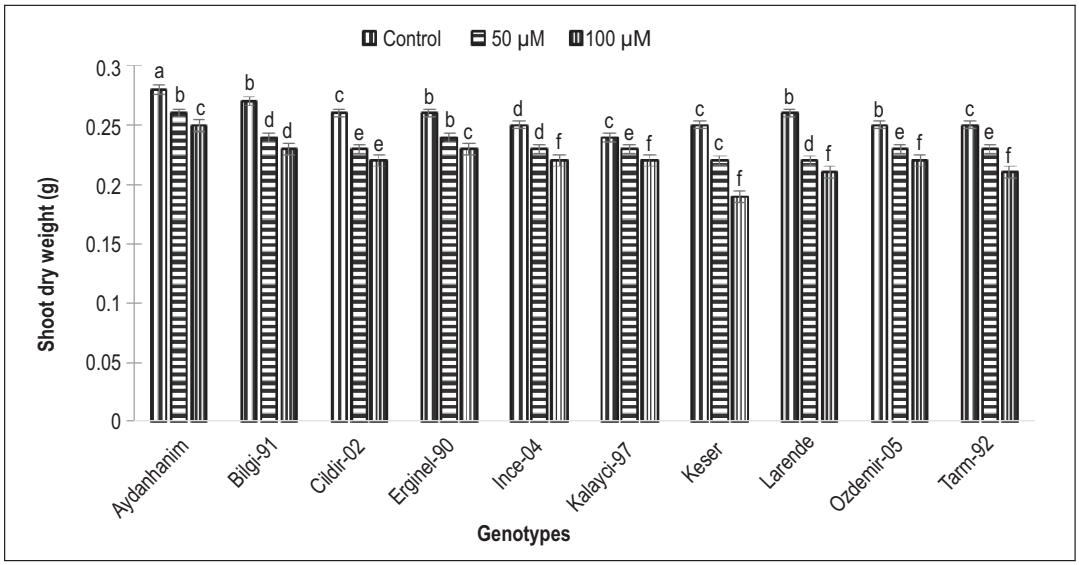


Fig. 5 : Shoot dry weight of 20-day-old seedlings of barley genotypes growing under Cd stress (0–100 µM). All values are means of three independent experiments with three replications each (n=9). Vertical lines indicate ± SE. The bars bearing different letters are statistically different at p≤ 0.5, as determined by the Duncan's Multiple Range Test.

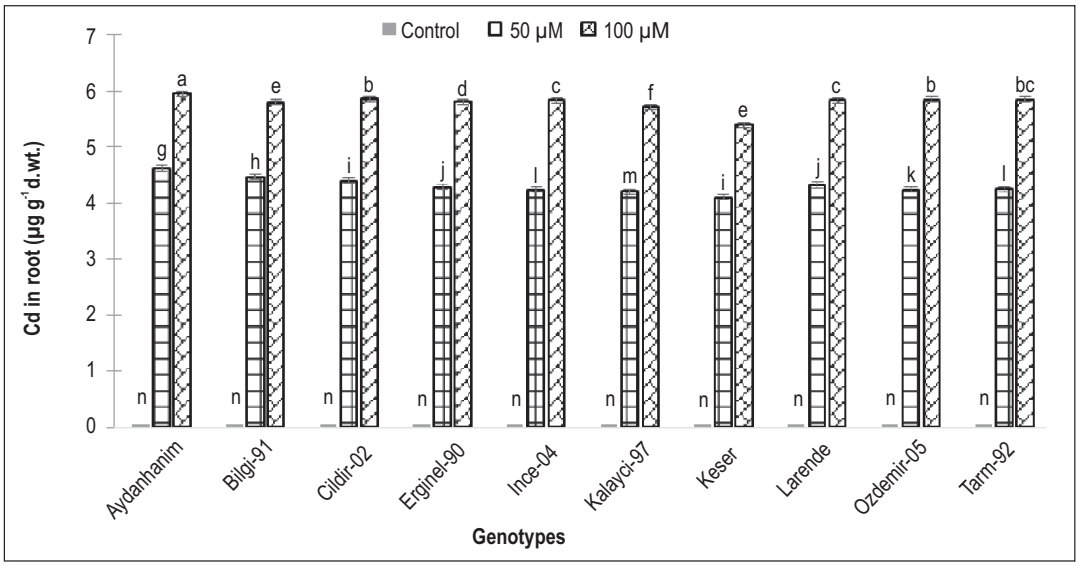


Fig. 6 : Cadmium accumulation in root of 20-day-old seedlings of barley genotypes growing under Cd stress (0–100 µM). All values are means of three independent experiments with three replications each (n=9). Vertical lines indicate ± SE. The bars bearing different letters are statistically different at p≤ 0.5, as determined by the Duncan's Multiple Range Test.

often lead to growth and yield restrictions (Khudsar *et al.,* 2000, 2001; Munzuroglu and Zengin, 2006). Growth arrest under high Cd stress (100 µM) can perhaps be linked to low mitotic activity or restricted cell enlargement due to decreased cellular turgor, as observed earlier in the root-tip meristem of *Vigna radiata* (Mumthas *et al.,* 2010). The inhibitory effect may also relate to the competition of Cd with essential metal ions, such as magnesium, iron, copper and zinc in the process of nutrient uptake, thus causing their deficiency in plants, which leads to growth impairment (Ali *et al.,* 1998a, 1998b; El-Kafafi and Rizk, 2013).

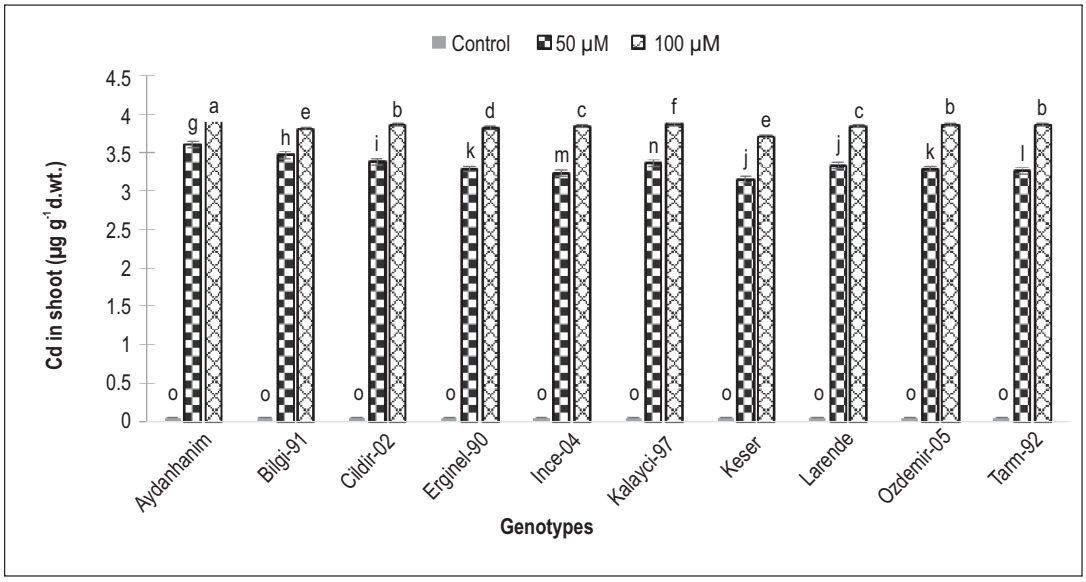


Fig. 7 : Cadmium accumulation in shoot of 20-day-old seedlings of barley genotypes growing under Cd stress (0–100 µM). All values are means of three independent experiments with three replications each (n=9). Vertical lines indicate ± SE. The bars bearing different letters are statistically different at p≤ 0.5, as determined by the Duncan's Multiple Range Test.

Cadmium application hampered plant biomass (d.wt.) in a concentration-dependent manner, showing the maximum effect at 100 µM. Root biomass was 0.38 g and 0.29 g in Aydanhanim whereas 0.35 g and 0.22 g in Keser exposed to 0 µM and 100 µM Cd, respectively (Fig. 4), which means an average reduction of 19% (Aydanhanim) to 40% (Keser) at 100 µM, compared to the control. In the case of shoot, the biomass was 0.28 and 0.25 g in Aydanhanim, whereas 0.25 g and 0.19 g in Keser, exposed to 0 µM and 100 µM Cd stress, respectively (Fig. 5). The average reduction due to 100 µM Cd, therefore, ranged from 11% (Aydanhanim) to 24% (Keser) as compared to their respective controls. Our observations conform to some earlier studies on *Brassica campestris* L., *Vigna radiata* L., and *Lactuca sativa* L. (Anjum *et al.,* 2008a; Mumthas *et al.,* 2010; Akhter *et al.,* 2014), among others. A decrease in biomass of plant parts might be due to inhibition of cell division and/or decrease in enzymatic activities (Mehindirata *et al.,* 1999).

Cd accumulation in roots and shoots of all the barley genotypes studied increased with increase in Cd concentration in the nutrient solution. In the control samples, all genotypes showed an insignificant and almost equal (0.5 µg g^{-1}) Cd concentration both in root and shoot tissues. It increased markedly under 50 and 100 µM Cd treatments. The genotypic capacity of Cd-accumulation under 100 µM stress was variable; ranging from 5.41 to 5.96 µg g^{-1} d.wt. in roots and 3.71 to 3.94 µg g^{-1} d.wt. in shoots of genotypes Keser and Aydanhanim respectively (Fig. 6 and 7). Thus, both in roots and shoots, the maximum accumulation occurred in Aydanhanim and minimum in Keser (Fig. 6 and 7). Translocation factor, defined as the ratio of Cd in shoots to Cd in roots, varied across the genotypes from 0.73 to 0.79 under 50 µM Cd and from 0.62 to 0.67 under 100 µM Cd treatments (Table 1). The largest translocation factor (0.79) was associated with Aydanhanim at both Cd treatments, while the lowest was recorded for Özdemir-05 and Tarm-92 at 50 µM and 100 µM treatments, respectively (Table 1). Cd level was higher in roots than in shoots, indicating that a higher proportion of Cd taken up by plants was sequestered to roots.

It is expected that the radial movement of Cd across the root may face some effective barriers such as the cells with the Casparian band. Moreover, in the case of barley, Cd

Table 1 : Translocation factor for ten barley genotypes exposed to 50 and 100 µM concentrations of Cd

	Cd concentration (µM)	
Genotype	**50**	**100**
Aydanhanim	0.79	0.67
Bilgi-91	0.78	0.66
Çildir -02	0.75	0.65
Erginel 90	0.77	0.64
Ince-04	0.76	0.66
Kalayci-97	0.78	0.63
Keser	0.74	0.65
Larende	0.77	0.66
Özdemir-05	0.73	0.66
Tarm-92	0.77	0.62

concentration may be higher in the symplast, indicating that barley immobilizes more Cd via chelation in the root, which would therefore reduce Cd transfer to shoots (Akhter *et al.,* 2014). Irrespective of the tissue type, most of the Cd^{2+} developed coordination bonds with S and SO_4 ligands in the barley roots. Panou-Filotheou and Bosabalidis (2004) opine that plants accumulate a higher Cd content in their roots than in shoots because roots are the first to contact Cd intimately, and Cd has a low mobility within the plant. Roots also provide a protection mechanism targeted at confining the metal perniciousness, thus defending the shoots from toxicity-caused disorders (Ansari *et al.,* 2009).

In conclusion, Turkish genotypes of barley mutually differ in the degree of seed germination, seedling growth and tolerance to Cd stress. Application of different Cd concentrations inhibited seed germination, dry mass accumulation and growth variables in barley genotypes, particularly in cv. Keser. A higher amount of Cd accumulation in the root of genotype Aydanhanim as well as its relatively better growth performance is suggestive of the genetic capability of this genotype to tolerate Cd stress, and retain and sequester Cd ions in roots. Thus, genotype Aydanhanim could possibly be used as a phytoremediator of Cd, as it possesses high tolerance potential and is, therefore, less susceptible to Cd-stress. However, the magnitude of the response of this genotype to Cd-stress must be evaluated under field conditions also.

Acknowledgments

The authors (IMA and MI) appreciate the International Science Partnership Program of King Saud University, Riyadh (Saudi Arabia) for financial assistance towards this study through ISPP # 00113. The first author (MKAA) thanks the Scientific and Technological Research Council of Turkey for awarding him with the Foreign Citizens Research Fellowship. We also thank the editor and reviewers for their wise suggestions.

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