TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2015) 39: 747-756

doi:10.3906/bot-1412-28

Random amplified polymorphic DNA as a method to screen metal-tolerant barley (*Hordeum vulgare* L.) genotypes

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Received: 16.12.2014 • **Accepted/Published Online:** 28.04.2015 • **Printed:** 30.09.2015

Abstract: Differentially responding genotypes are comparatively used to understand mechanisms of metal tolerance in higher plants. The feasibility of the random amplified polymorphic DNA (RAPD) technique was evaluated to screen for metal tolerance within plants as an alternative to physiological growth parameters. Eight hydroponically grown barley (*Hordeum vulgare* L.) genotypes were exposed to different concentrations (75, 150, and 225 µM) of cadmium and copper for 7 days and subsequently harvested for determining metal tolerance indices by comparing dry biomass to genomic template stabilities based on their RAPD profiles. Dose- and genotype-dependent reductions in dry weights of shoot and root tissues were detected for both metal treatments. The tolerance indices showed that Aydanhanım/Bülbül-89 and Başgül/Tarm-92 were the most tolerant/sensitive barley genotypes to cadmium and copper, respectively. For untreated barley genotypes, the amplified RAPD bands were genotype-dependent and estimated genetic similarity coefficients ranged from 0.56 to 0.82. Metal exposure caused appearance and/or disappearance of RAPD bands in comparison to the control RAPD profiles. The alterations in RAPD profiles were dose- and genotype-dependent for the cadmium and copper treatment groups. Consequently, genomic template stability (GTS, %) of all barley genotypes was reduced with metal exposure. Based on GTS values, the most tolerant/sensitive barley genotypes to cadmium and copper were Aydanhanım/Tarm-92 and Avcı-2002/Orza-96, respectively. The growth and RAPD results clearly indicated that Aydanhanım, Avcı-2002, and Başgül were more tolerant, while Bülbül-89, Orza-96, and Tarm-92 were more sensitive barley genotypes to both metals. The RAPD technique could be used to screen metal-tolerant plant genotypes as an alternative method.

Key words: Barley, cadmium, copper, genomic template stability, tolerance index

1. Introduction

The higher plants absorb numerous elements from the soil and the air through their roots and their leaves, respectively. Some essential elements such as iron, copper, manganese, molybdenum, nickel, and zinc are referred to as micronutrients because they are required by plants in minute quantities (Peralta-Videa et al., 2009). It has been documented elsewhere that plants also absorb toxic metals such as cadmium, lead, and mercury that have no known biological function for higher plants (Gratão et al., 2005). The bioavailability of heavy metals (e.g., cadmium) at any concentration or micronutrients (e.g., copper) at higher concentrations in the soil leads to the accumulation of these metals in the plant roots and leaves (Sytar et al., 2013). Numerous studies have clearly indicated that exposing the plants to cadmium or excess copper inhibits seed germination, plant root/shoot growth, and photosynthetic machinery; reduces chlorophyll content; (in)activates enzymes; alters gene expression; and induces lipid peroxidation (Munzuroglu and Geckil, 2002; Pereira et al., 2002; Monteiro et al., 2011). The accumulated cadmium or copper in various parts of plants also induces cellular stress responses and damage to different cellular components (such as membranes, proteins, and DNA) and ultrastructural changes (Yruela, 2005; Gratão et al., 2009). The damaging effects of cadmium and/or copper on the genome of the organisms have been clearly indicated by using various molecular techniques including chromosomal aberrations (Ünyayar et al., 2006), the Ames test (Codina et al., 1995), comet assays (Hattab et al., 2009), and DNA fingerprinting. DNA fingerprinting has been frequently used to show the level of DNA alterations in genotoxin-exposed organisms since being first introduced by Savva (1996).

Random amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR)-based and quite reproducible (if the conditions are set up properly) DNA fingerprinting technique that yields information on a large number of markers without having to obtain DNA sequence information for primer design (Agrawal et al., 2008).

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RAPD has been commonly used for a variety of purposes such as cultivar identification, genetic diversity assessment, and the construction of phylogenetic relationships (Cenkci et al., 2008), and it has been successfully utilized in genotoxicity judgment of suspicious chemicals (Cenkci et al., 2009). DNA alterations in the genome can clearly be shown by comparing DNA fingerprints from untreated and treated individuals to genotoxic agents (Atienzar and Jha, 2006; Aras et al., 2012). The differences in the RAPD profiles could also be used to estimate reduction rates in the genomic DNA template stability (GTS, %) that are comparable with changes in some physiological parameters such as root growth, total soluble protein content, and chlorophyll pigment contents (Atienzar et al., 2001; Cenkci et al., 2010b). On the other hand, there are no (to our knowledge) or few (possibly) reports on the screening of metal-tolerant plant cultivars by using the RAPD or a similar molecular marker technique.

Plant genotypes differentially respond to metal toxicity at physiological, biochemical, and molecular levels (Metwally et al., 2005). Metal-tolerant and metal-sensitive plant genotypes are comparatively used to understand mechanisms of metal tolerance in higher plants (Ekmekçi et al., 2008). We aimed to investigate the potential of molecular markers to screen or monitor metal-tolerant and metal-sensitive plant genotypes as an alternative to physiological parameters. Therefore, in the present study, we have determined cadmium and copper tolerances of eight barley (*Hordeum vulgare* L.) cultivars by using both the growth and RAPD parameters in order to show the viability of the RAPD technique in determining how plant genotypes differentially respond to metal toxicity.

2. Materials and methods

2.1. Plant material and growth conditions

The selected seeds of eight barley (*Hordeum vulgare* L.) genotypes (Angora, Avcı-2002, Aydanhanım, Başgül, Bülbül-89, Orza-96, Tarm-92, and Zeynelağa; obtained from the Ankara Field Crops Central Research Institute of Turkey) were dipped in 5% aqueous (v/v) sodium hypochlorite for 10 min, rinsed three to four times with distilled water, and then soaked in distilled water for 3 h for imbibition. The barley seeds were germinated on two germination papers wetted with distilled water for 3 days in the dark and at 25 ± 1 °C. Three-day-old etiolated barley seedlings were transferred to ½ Hewitt's nutrient solution supplemented with preliminary determined concentrations (75, 150, and 225 μM) of $CdCl_2$ or $CuSO_4$ and grown hydroponically. The control and contaminated (including cadmium or copper) nutrition was refreshed every 2 days in order to maintain the pH of nutrient solutions in a range of 6.3–6.6 and to facilitate the root aeration. The seedlings were cultured in a growth chamber at 260 μmol m^{-2} s^{-1} light intensity for a 12-h photoperiod at 25 ± 1 °C for 7 days.

2.2. Cadmium and copper tolerance indices

After harvesting, the roots of each seedling were rinsed three times in distilled water to remove excess metals from root surfaces. The shoot and root tissues of each replicate (the tissues of 12 seedlings) were separated and dried in an oven at 80 °C for 48 h. The dry weights of shoot and root were calculated per plant with standard errors (±SE). The tolerance index value (%) for each cadmium and copper treatment was calculated as a ratio between the dry mass of shoots or roots grown in the solution with cadmium or copper and that of plants grown in the control solution (Bağcı et al., 2003). The tolerance index was then scored for each treatment by assigning values from 1 to 8 to each treatment, with increasing scores indicative of increasing tolerance. The scores of a barley genotype exposed to different metal concentrations were added, and the final scores were used to categorize the genotypes for their tolerance to cadmium and copper.

2.3. RAPD analysis

The genomic DNA was extracted from root tips of five seedlings for each treatment group as described in Cenkci et al. (2010b). DNA concentrations and integrity were estimated spectrophotometrically (TU-1880 Double Beam UV-VIS). PCR reactions were performed in reaction mixtures of 50 μL containing 50 ng of genomic DNA, 2 μM primer (Operon Technologies, Inc., Alameda, CA, USA), 200 μM dNTPs (50 μM each), 1X PCR buffer, and 1.5 U of Taq DNA polymerase (Thermo Fisher Scientific, USA). A total of 20 RAPD primers were initially screened and the following primers were selected to be used in the RAPD analyses: OPA01 (CAGGCCCTTC), OPA02 (TGCCGAGCTG), OPA09 (GGGTAACGCC), OPA10 (GTGATCGCAG), OPA12 (TCGGCGATAG), OPA16 (AGCCAGCGAA), OPA17 (GACCGCTTGT), and OPA19 (CAAACGTCGG). Amplifications were performed in a DNA thermocycler (Uvigene, Uvitech Ltd., UK) programmed for 4 min at 94 °C for initial denaturing and 40 consecutive cycles of 45 s at 94 °C for denaturing, 45 s at 37 °C for annealing, and 60 s at 72 °C for polymerization. The cycling was completed by a final extension step for 8 min at 72 °C. RAPD-PCR products were separated on 1.8% agarose gels by electrophoresis. The GeneRuler 100bp DNA Ladder Plus (Thermo Fisher Scientific) was used as the molecular weight DNA standard. The ethidium bromide-stained DNA bands were visualized using a UV transilluminator. The size of each amplification product was automatically estimated using the UVIsoft image analyzer system.

Reproducible RAPD bands were scored and used to compile a data matrix for each barley cultivar. A unique control RAPD profile was used for both cadmium and

copper treatments. The obvious alterations (disappearance and/or appearance of bands) detected in RAPD patterns for each treatment were evaluated for each primer in comparison with their control RAPD profiles. Each change observed in RAPD patterns (disappearance of a present band or appearance of new band) was given the arbitrary score of +1, and the average was calculated for each experimental group with the primers tested. The similarity coefficients among untreated barley genotypes were estimated from Nei's unbiased measure (Saitou and Nei, 1987) using Jaccard's algorithm. Neighbor-joining (NJ) trees after 1000 replicate bootstrap tests were generated from the matrix for untreated barley cultivars using the FreeTree (Pavlicek et al., 1998) and TreeView (Page, 1996) software packages. The genomic template stability (GTS, %) was calculated for each primer by the formula (100 × a/n), where *n* is the number of bands selected in the control RAPD profile and *a* is the average number of band changes in DNA profiles of the treatment group (Atienzar et al., 1999). To rank the sensitivity of the measured parameters (GTS and tolerance index), changes in these values were calculated as a percentage of their control value (set to 100%). The value of GTS was then scored for each treatment by assigning values from 1 to 8 to each treatment, with increasing scores indicative of increasing tolerance. Final scores were used to categorize the genotypes for their tolerance to cadmium and copper.

2.4. Statistical analysis

All data presented are the mean values with standard errors (±SE) of three independent replicates with 12 plants each. Statistical analysis was carried out by one-way ANOVA using Duncan's test to determine the significance of the differences between means. Means were considered significantly different at $P < 0.05$.

3. Results

The exposure of cadmium or copper (75, 150, and 225 µM) to eight barley (*Hordeum vulgare* L.) genotypes significantly inhibited the seedling growth in terms of elongation and fresh/dry biomass accumulation in the shoot and root tissues. The effect of genotype and metals (cadmium or copper) or their interactions had significant effects on dry weight accumulations ($P < 0.05$). The 75 µM cadmium treatment significantly reduced the shoot and root dry weights in all tested barley genotypes in comparison to their controls ($P < 0.05$, Figure 1). Further significant reductions were also observed in shoot dry weights of a majority of genotypes with an increase in cadmium concentrations (75 µM to 225 µM). In general, dry root weights were gradually decreased with an increase in cadmium concentrations, and some of these reductions were significant ($P < 0.05$) (Figure 1). Similarly, a sharp decrease in shoot and root dry weights occurred for all barley genotypes after 75 µM copper exposure as compared with their controls (Figure 2). Shoot dry weights of the majority of barley genotypes were significantly reduced after exposure to the 150 µM copper treatment in comparison to the lower treatment. However, there was no significant reduction in the dry weights after increasing the copper concentrations from 75 µM to 225 µM, even though gradual decreases were observed in all measured weights (Figure 2). The calculated shoot to root dry weight ratios of barley genotypes were slightly decreased in both cadmium and copper treatments in comparison to their controls (data not shown). In order to define the tolerance level of each barley genotype exposed to cadmium or copper, tolerance index values (%) were calculated from the data of shoot and root dry weights. Cadmium and copper tolerant indices with their scores are presented in Figures 3 and 4, respectively. Cadmium tolerance indices varied depending on barley genotype, from 54.6% to 91.3% and from 52.3% to 87.5% for the shoot and root dry weights, respectively. In the case of copper treatment, tolerance indices varied from 47.8% to 62.4% and from 56.0% to 90.0% for the shoot and root dry weights, respectively. Taking into consideration the score of tolerance index values, the eight barley genotypes were ranked with respect to their tolerance to cadmium: Aydanhanım > Avcı-2002 > Zeynelağa > Angora ≥ Başgül > Tarm-92 > Orza-96 > Bülbül-89 (Figure 3). The order of tolerant barley genotypes on the basis of copper tolerance indices was Başgül > Avcı-2002 > Aydanhanım > Zeynelağa > Angora > Bülbül-89 > Orza-96 > Tarm-92 (Figure 4).

Aimed at determining tolerance levels of barley genotypes by the RAPD technique, root tips of five barley seedlings (~200 mg) representing each experimental group were used to extract genomic DNA as described by Cenkci et al. (2010b). Twenty 10mer oligonucleotide primers of 60%–70% GC content were utilized to screen barley genomes for alterations after cadmium and copper exposure, but only eight primers generated informative results. In summary, 92 different RAPD bands (11.5 band per primer) ranging from 141 (OPA02) to 2243 (OPA16) bp were identified in RAPD profiles of untreated controls for all barley genotypes. Of these bands, 32 were monomorphic (34.8%) while the remainder were polymorphic (65.2%). The estimated genetic similarity coefficients among untreated barley genotypes ranged from 0.56 (between genotypes Angora and Avcı-2002) to 0.82 (between genotypes Orza-96 and Tarm-92). The 67 NJ trees after 1000 replicate bootstrap tests were generated from the matrix. The most frequent NJ tree clustered the barley genotypes in three subgroups (Figure 5). The genotype Avcı-2002 was grouped alone; Zeynelağa, Aydanhanım, and Angora were grouped together; and, finally, the Tarm-92/Orza-96 and Bülbül-89/Başgül pairs were clustered together.

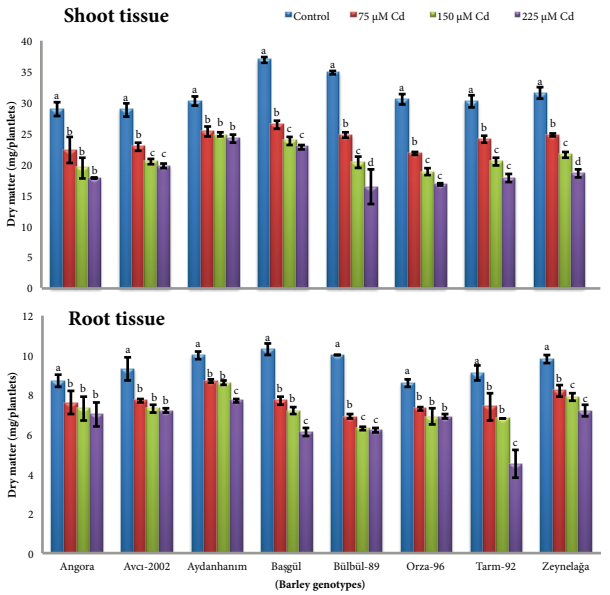

Figure 1. The effect of 7-day exposure of different cadmium (Cd) concentrations on the shoot and root dry weights of eight barley genotypes. The *a–d* letters on the standard error bars indicate significant ($P < 0.05$) differences among the mean values. Each value is the mean of three replicates with a total of 12 plantlets ($n = 3$).

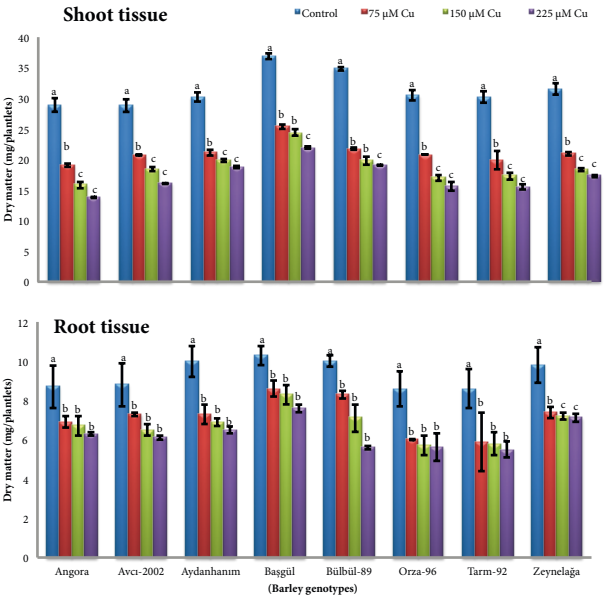

Figure 2. The effect of 7-day exposure of different copper (Cu) concentrations on the shoot and root dry weights of eight barley genotypes. The *a–c* letters on the standard error bars indicate significant ($P < 0.05$) differences among the mean values. Each value is the mean of three replicates with a total of 12 plantlets ($n = 3$).

On the other hand, exposure of cadmium and copper to the barley genotypes caused the appearance and/or disappearance of some RAPD bands in comparison to their controls. Tables 1 and 2 summarize these changes with calculated GTS values for cadmium and copper treatments, respectively. Only a few band changes were recorded in the RAPD profiles of Aydanhanım, Avcı-2002, and Başgül exposed to the various concentrations (75, 150 and 225 µM) of cadmium and copper. However, a higher number of DNA band changes in comparison to their controls was detected in cadmium- or copper-exposed Bülbül-89, Orza-96, and Tarm-92. Figure 6 represents the RAPD profiles of cadmium- and copper-tolerant/sensitive barley genotypes generated by primer OPA-02. The GTS, a qualitative measure reflecting changes in RAPD profiles of cadmium- and copper-treated barley cultivars were calculated for each of the eight primers tested (Tables 1 and 2). The barley genotypes were ranked on the basis of genomic template stability to cadmium, giving results of Aydanhanım > Avcı-2002 ≥ Başgül > Angora > Zeynelağa > Orza-96 > Bülbül-89 > Tarm-92. The eight barley genotypes were in addition ranked on the basis of GTS with respect to their tolerance to copper, giving results of Avcı-2002 > Aydanhanım > Başgül > Bülbül-89 > Angora ≥ Zeynelağa > Tarm-92 > Orza-96.

4. Discussion

Mechanisms of tolerance help plants and algae to maintain growth even in the presence of potentially toxic metal concentrations (Clemens, 2006). The exposure of plants to higher concentrations of metals induces a wide range of physiological and metabolic alterations (Gratão et al., 2005; Hossain et al., 2012). In the present study, the seedlings of eight barley genotypes were exposed to different concentrations (75, 150, and 225 µM) of cadmium and copper. In total, shoot and root dry weights of 2304 seedlings (the measurements of aborted experiments not included) were determined after harvesting in order to obtain data for interpreting the metal tolerance of barley cultivars. Dose-dependent reduction in shoot and root dry weights of all tested barley cultivars exposed to cadmium or copper was observed. There are many reports demonstrating that application of cadmium or copper reduces dry biomass accumulation in a dose-dependent manner (Gratão et al., 2005; Bauddh and Singh, 2011; Körpe and Aras, 2011; Monteiro et al., 2011; Kalai et al., 2014). However, the treatment with lower concentrations of cadmium, boron, or nickel could induce the root and seedling growth in plants and consequently could lead to higher biomass accumulation (Gratão et al., 2008). The growth inhibition in cadmium-treated barley seedlings may be due to the interference of vital metabolic processes such as photosynthesis, respiration, and nutrient transport

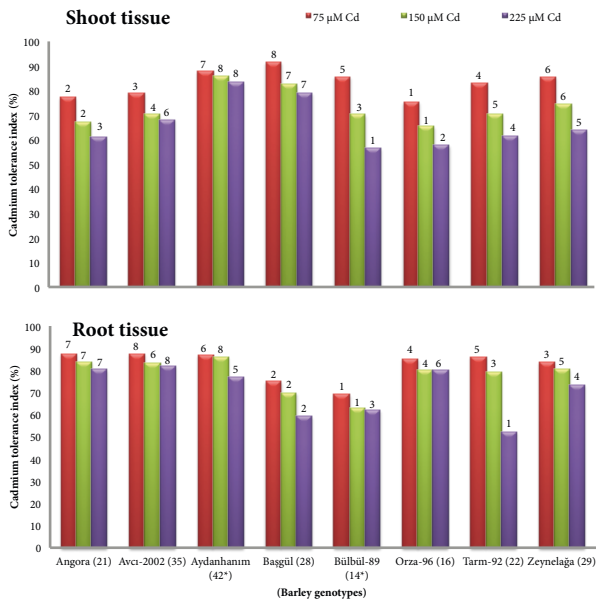

Figure 3. Cadmium (Cd) tolerance index values (%) and their scores (given on the bars) for eight barley genotypes determined based on shoot and root dry weights. The total scores are given under barley genotypes in parentheses. The highest and the lowest total scores indicate the least and the highest tolerance to cadmium exposure, respectively.

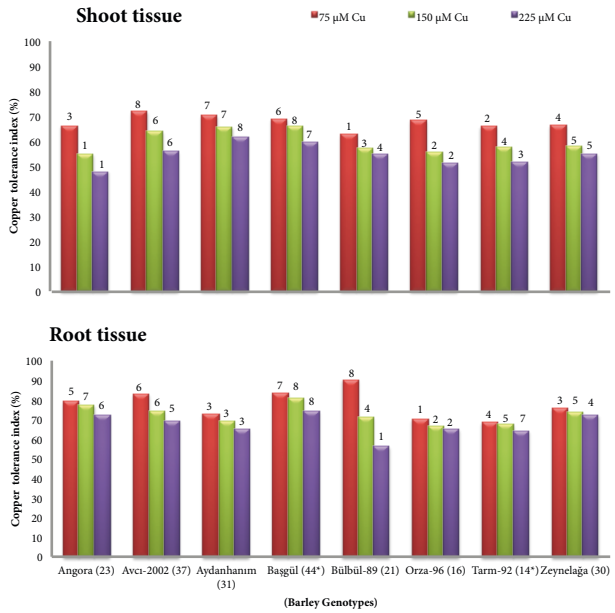

Figure 4. Copper (Cu) tolerance index values (%) and their scores (given on the bars) for eight barley genotypes determined based on shoot and root dry weights. The total scores are given under barley genotypes in parentheses. The highest and the lowest total scores indicate the least and the highest tolerance to copper exposure, respectively.

(Gill et al., 2011). Similarly, excess copper causes injury to plants and inhibition of cell elongation and cell division, which ultimately leads to retardation of plant growth (Körpe and Aras, 2011; Kalai et al., 2014). As an inevitable consequence, stunted shoot and root growth is one of the physiological end-points that can be a measure of exposure to toxic amounts of metals.

Plants differentially respond to metal toxicity and different plant cultivars may have different levels of tolerance due to their different genetic potentials (Bauddh and Singh, 2011). The heavy metal tolerance of a plant genotype might be related to its metal avoidance or metal accumulation capacity (Mehes-Smith et al., 2013). A hydroponic screening of 22 grass species indicated that barley together with oats (*Avena sativa*) is one of the more tolerant grass species to high cadmium and copper toxicity (Ebbs and Kochian, 1998). Shoot and root lengths are not always related to shoot and root weights in barley genotypes, as indicated in the report by Bağcı et al. (2003). Therefore, in the present study, the cadmium and copper tolerance indices were estimated based on shoot and root dry weights. Aydanhanım/Bülbül-89 and Başgül/Tarm-92 were the most tolerant/sensitive barley genotypes to cadmium and copper toxicity, respectively. The metal tolerance index is frequently used as a tool to screen tolerant plant genotypes against abiotic stresses such as toxic metals and salt. Indeed, there is no unique parameter to determine metal or salt tolerance of plant genotypes. For instance, the shoot and root dry weights (Bağcı et al., 2003; Gill et al., 2011), root lengths (Roy and Bhadra, 2014), shoot and root lengths (Bauddh and Singh, 2011), shoot fresh weights (Jaarsma et al., 2013), and shoot and root fresh weights (Metwally et al., 2005) have all been used to evaluate metal or salt tolerance of plant genotypes. There is no standard way to calculate tolerance index for plant species or cultivars against metal exposure. However, one of the major difficulties in studies on the selection of heavy metal-tolerant plants is the proper methodology that must ensure an efficient evaluation of a large number of plants, but reducing environmental contamination (Piotto et al., 2014). Therefore, as an alternative to physiological parameters, we have attempted to select tolerant or sensitive plant cultivars to metal toxicity by using the molecular marker-based RAPD technique.

The RAPD technique has been developed into a powerful tool to analyze genetic relationships and genetic diversity among economically important plant cultivars. In this study, the numbers and/or sizes of amplified RAPD-PCR bands were genotype-dependent for untreated barley cultivars. The estimated genetic similarity coefficients among untreated barley genotypes ranged from 0.56 to 0.82. This wide range demonstrates the process of analysis of genotypes with different degrees of dissimilarity, as found in other collections of barley accessions as shown with RAPD (Tanyolaç, 2003; Amabile et al., 2014).

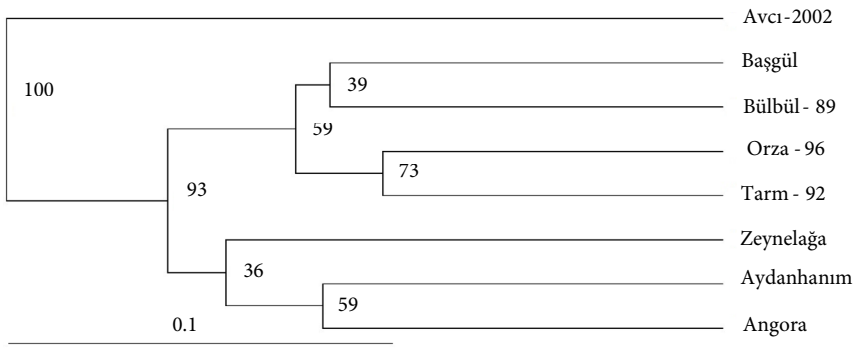

Figure 5. Cluster analysis of RAPD data for eight untreated barley genotypes. The neighbor-joining tree by the RAPD method is given as a phylogram. The percentages of replicate trees in the bootstrap test (1000 replicates) are shown next to the branches.

Table 1. The control (C), newly appeared (+), and disappeared (-) RAPD bands for each primer and calculated genomic template stability (GTS) values with their scores for each barley genotype exposed to different cadmium (Cd) treatments.

Genotypes	Cd, µM	OPA01			OPA02			OPA09			OPA10			OPA12			OPA16			OPA17			OPA19			GTS,%	Scores
		C	+	-	C	+	-	C	+	-	C	+	-	C	+	-	C	+	-	C	+	-	C	+	-		
Angora	75	9	0	0	12	1	0	8	0	0	8	0	0	4	0	0	9	0	0	17	0	0	9	0	0	98.9 (7)	16
	150		0	0		1	0		0	0		0	0		2	0		0	0		0	0		0	0	92.7 (4)	
	225		0	0		2	0		1	0		0	0		2	0		0	0		0	0		0	0	90.1 (5)	
Avcı-2002	75	14	0	0	11	0	0	11	0	1	10	0	1	4	0	0	14	1	1	11	0	0	14	0	0	95.9 (5)	17
	150		0	0		0	0		0	1		0	1		0	0		1	1		1	0		1	0	94.1 (5)	
	225		0	0		0	0		0	1		0	1		0	0		1	1		2	0		2	0	92.3 (7)	
Aydanhanım	75	11	0	0	9	0	0	9	0	0	14	0	0	6	0	0	11	0	0	17	0	0	10	0	0	100.0 (8)	24
	150		0	0		0	0		0	0		0	0		1	0		0	0		0	0		0	0	97.9 (8)	
	225		0	0		0	0		0	0		0	0		1	0		1	0		0	0		1	0	95.7 (8)	
Başgül	75	12	1	0	8	0	0	10	1	0	10	0	0	5	0	0	13	2	0	15	0	0	9	0	0	95.8 (4)	17
	150		1	0		0	0		1	0		0	0		0	0		2	0		0	0		0	0	95.8 (7)	
	225		1	0		1	0		2	0		0	0		0	0		2	0		1	0		0	0	91.6 (6)	
Bülbül-89	75	13	0	0	11	3	0	9	0	0	10	0	0	4	2	0	15	1	0	12	0	0	11	0	0	89.5 (2)	6
	150		0	0		3	0		0	0		0	0		2	0		1	0		2	1		1	0	86.5 (2)	
	225		0	0		4	0		3	0		0	0		2	0		1	0		2	1		1	1	80.1 (2)	
Orza-96	75	14	0	0	7	2	0	8	1	1	7	0	0	5	0	0	13	1	0	14	0	0	13	2	0	90.4 (3)	9
	150		0	0		2	0		2	0		0	0		0	0		1	0		0	0		2	1	89.4 (3)	
	225		0	0		2	0		2	0		0	0		0	0		1	0		0	1		2	1	88.5 (3)	
Tarm-92	75	14	0	0	7	6	0	7	3	0	9	1	0	8	2	0	9	4	0	11	0	0	11	0	0	72.7 (1)	3
	150		0	0		6	0		4	0		2	0		2	0		4	0		1	0		0	0	68.8 (1)	
	225		0	0		6	0		4	0		2	0		2	0		5	0		1	0		0	0	66.4 (1)	
Zeynelağa	75	12	0	0	12	1	0	11	0	1	10	0	0	9	0	0	16	0	0	18	0	0	10	0	1	96.6 (6)	10
	150		0	0		1	0		0	0		0	0		2	0		0	0		0	0		0	1	94.9 (6)	
	225		0	0		2	0		0	0		1	0		2	2		0	1		0	0		0	1	89.1 (4)	

Table 2. The control (C), newly appeared (+) and disappeared (-) RAPD bands for each primer and calculated genomic template stability (GTS) values with their scores for each barley genotype exposed to different copper (Cu) treatments.

Genotypes	Cu, µM	OPA01			OPA02			OPA09			OPA10			OPA12			OPA16			OPA17			OPA19			GTS,%	Scores
		C	+	-	C	+	-	C	+	-	C	+	-	C	+	-	C	+	-	C	+	-	C	+	-		
	75	9	0	0	12	1	1	8	0	1	8	0	0	4	0	0	9	0	0	17	0	1	9	0	1	94.2 (3)	
Angora	150		3	0		1	1		1	1		0	0		0	0		0	0		0	1		1	1	87.1 (3)	10
	225		3	0		1	1		1	1		0	0		0	0		0	0		0	2		1	1	86.3 (4)	
	75	14	0	0	11	0	0	11	0	0	10	0	0	4	0	0	14	0	1	11	0	0	14	0	0	99.1 (8)	
Avcı-2002	150		0	0		0	1		0	0		0	0		0	0		0	1		0	1		0	0	97.2 (7)	23
	225		0	0		0	1		0	0		0	0		0	0		2	1		0	1		0	0	95.4 (8)	
	75	11	0	0	9	0	0	9	0	0	14	0	0	6	0	0	11	0	1	17	0	0	10	0	0	99.1 (7)	
Aydanhanım	150		0	0		0	0		0	0		0	0		0	0		0	1		0	0		0	0	99.1 (8)	20
	225		0	0		0	0		1	0		0	0		0	1		2	1		0	0		0	0	93.9 (5)	
	75	12	0	0	8	1	0	10	0	0	10	0	0	5	0	0	13	1	0	15	0	0	9	0	0	96.9 (6)	
Başgül	150		0	0		1	0		0	0		0	0		0	0		1	0		0	0		0	0	96.9 (6)	19
	225		0	0		1	1		0	0		0	0		0	0		1	0		0	0		0	0	94.8 (7)	
	75	13	0	0	11	1	0	9	0	0	10	0	0	4	1	0	15	0		12	0	0	11	0	0	95.7 (5)	
Bülbül-89	150		0	0		1	0		0	0		0	0		1	0		0	0		0	0		0	1	95.2 (5)	16
	225		0	0		1	0		1	0		0	0		1	0		1	0		0	0		0	0	94.1 (6)	
	75	14	0	0	7	2	0	8	1	0	7	0	0	5	1	0	13	0	0	14	0	0	13	0	0	92.4 (1)	
Orza-96	150		0	0		6	0		1	0		0	0		1	0		1	1		0	4		0	0	81.5 (1)	3
	225		0	0		6	0		1	0		3	0		1	0		1	1		0	4		0	0	76.2 (1)	
	75	14	0	0	7	0	0	7	1	1	9	0	0	8	0	0	9	2	0	11	0	0	11	0	0	93.3(2)	
Tarm-92	150		0	0		0	0		3	1		0	2		0	2		2	0		0	0		0	0	83.4 (2)	7
	225		0	0		0	0		3	1		0	2		0	2		2	0		0	0		0	0	83.4 (3)	
	75	12	0	0	12	1	0	11	0	0	10	0	1	9	0	2	16	0	0	18	0	1	10	0	0	94.2 (4)	
Zeynelağa	150		0	0		1	0		0	1		0	1		0	2		0	0		0	2		0	0	92.4 (4)	10
	225		0	0		2	0		0	3		0	3		0	5		0	0		0	2		0	0	82.4 (2)	

Nevertheless, the aim of this study was to screen metal-tolerant and metal-sensitive barley cultivars by the RAPD technique rather than genetic analysis of barley genotypes. In the present study, the genomic DNAs were extracted from root tissues of untreated and treated barley genotypes due to the fact that metal genotoxicity is more evident in the roots than other parts of plants (Cenkci et al., 2009). RAPD profiles of treated and untreated groups are used to determine the level of genotoxicity in plants (Aras et al., 2012). The exercise of exposing eight barley genotypes to various concentrations of cadmium and copper resulted in the appearance and/or disappearance of some RAPD bands in comparison to those of untreated barley genotypes. These findings clearly indicated that both metals cause genotoxic effects on the roots of barley genotypes. Cadmium and copper induce DNA damage (e.g., single- and double-strand breaks, modified bases, abasic sites, DNA-protein cross-links, oxidized bases, 8-hydroxyguanine, and bulky adducts), mutations and/or complex chromosomal rearrangements in organism genomes (Waisberg et al., 2003; Mukherjee et al., 2004). The presence of DNA damage and mutations in the genome could block or reduce (bypass event) polymerization of DNA in the PCR reaction (Atienzar and Jha, 2006). Therefore, the loss of control RAPD bands and newly appeared bands detected in the RAPD profiles of treatment groups are most probably because of these alterations. In comparison to the control RAPD profiles, the largest changes in RAPD profiles (disappeared and/or appeared bands) were detected at 225 µM cadmium and copper treatment for all barley genotypes. Dose-dependent genotoxicity of cadmium and copper was also

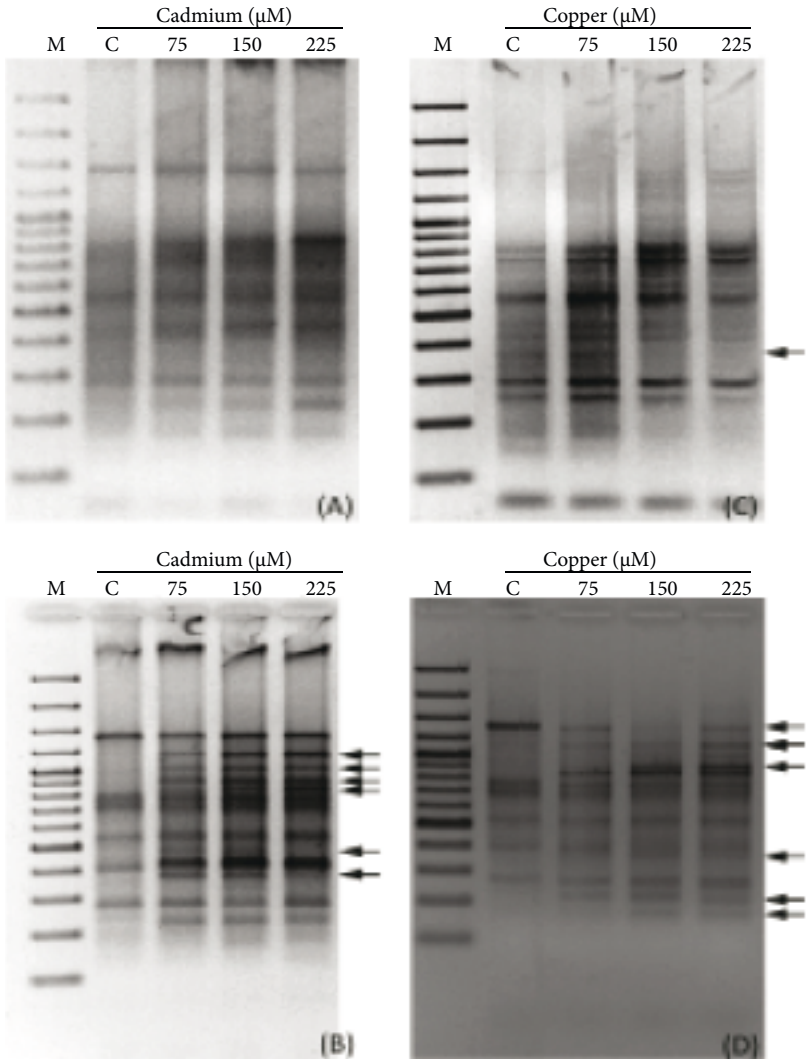

Figure 6. RAPD profiles of cadmium-tolerant Aydanhanım (A), cadmium sensitive-Tarm-92 (B), copper-tolerant Avcı-2002 (C), and copper-sensitive Orza-96 (D) after control (C), 75, 150, and 225 µM treatments. RAPD profiles were generated using primer OPA02. M: GeneRuler 100 bp plus DNA Ladder (100–3000 bp). The arrows indicate appeared and/or disappeared RAPD bands in the treatment groups as compared to their control RAPD profiles.

revealed by the RAPD technique in various plant genomes including barley (*Hordeum vulgare* L.), eggplant (*Solanum melongena* L.), and kidney bean (*Phaseolus vulgaris* L.) (Liu et al., 2005; Enan, 2006; Körpe and Aras, 2011).

It was demonstrated that differences between RAPD profiles of untreated and treated samples could also be regarded as modifications in GTS (Atienzar et al., 1999). The GTS value as a qualitative measure of genotoxic effect has been directly used to compare with alterations in other parameters such as root elongation, protein content, comet assay, and pigment content (Liu et al., 2005, 2007; Cenkci et al., 2010a; Körpe and Aras, 2011). In present study, eight different barley genotypes were evaluated in RAPD experiments after exposing them to different concentrations of cadmium and copper. The barley genotypes were differentially affected by the cadmium and copper genotoxicity treatments. The genomic template stabilities were calculated for each treatment group of the barley genotype on the basis of each primer. The results indicated a genotype-dependent decrease in genomic template stabilities for both cadmium and copper treatments. Based on GTS values, Aydanhanım/Tarm-92 and Avcı-2002/Orza-96 were the most tolerant/sensitive barley genotypes to cadmium and copper genotoxicity, respectively.

Following exposure to increasing cadmium and copper,

the metal tolerance index and GTS of the eight barley genotypes decreased gradually, but the growth parameters seem more sensitive than RAPD profiles. The growth parameters in all barley genotypes were significantly inhibited after 75 µM cadmium or copper exposure. The correlation analyses between tolerance index based on physiological parameters and GTS suggest that the level of GTS accuracy could be used to determine different levels of Cd and Cu tolerance. In contrast, the reduction in the GTS was more obvious in the 225 µM metal treatments. In agreement with our results, the GTS was found to be less sensitive than growth parameters in similar RAPD studies reported by Atienzar et al. (1999), Liu et al. (2005), and Cenkci et al. (2010a, 2010b). However, the obvious effects of genotoxins on studied genomes can be easily followed at higher metal concentrations as shown in this study. The growth and RAPD data indicated that barley genotypes differentially respond to cadmium and copper toxicity, and a similar, but not exact, match of tolerance categorizations were obtained using physiological and molecular techniques. Moreover, the lengths and dry/fresh weights of a few thousand barley seedlings were measured with rough rulers and analytical scales after long and tedious laboratory work. In contrast, the RAPD method is quick, involves less labor, is PCR-based, is indicative of DNA stability, and requires only few hundred barley seedlings.

In conclusion, the tolerance levels of eight barley genotypes to cadmium and copper were determined on the basis of physiological and molecular parameters. The growth and RAPD results indicated that Aydanhanım, Avcı-2002, and Başgül were more tolerant, while Bülbül-89, Orza-96, and Tarm-92 were more sensitive barley genotypes to metal exposure. Therefore, the RAPD technique could be used to screen metal-tolerant genotypes in higher plants.

Acknowledgment

The authors wish to thank the Afyon Kocatepe University Research Fund (Project No: 11.FENED.08 and 11.FENBİL.11) for financial support.

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