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Genotypic Differences in Effect of Cd on Growth and Mineral Concentrations in Barley Seedlings

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Cadmium (Cd) has become a widespread pollutant in agricultural soils mainly due to industrial emission, the application of sewage sludge and phosphate fertilizers containing Cd (Davis, 1984). Cd can be taken up by plant roots and translocated to above-ground tissues (Yang *et al.,* 1998), and then becomes a potential threat to human health as it enters the food chain (Obata and Umebayashi, 1997). Such a situation did occur in the 1950s and 1960s in Japan where Cd contamination of rice fields led to renal impairment and bone disease in exposed populations. The World Health Organization (WHO) set a maximum provisional tolerable intake limit for an adult at 60 to 70 μg Cd per day (WHO, 1972) and the Codex Alimentary Commission of FAO/WHO is discussing a limit of 0.1μg Cd g^{-1} for cereal grains traded on international markets. Cd toxicity has turned into a potential agricultural and environmental issue worldwide (Obata and Umebayashi, 1997; Davis, 1984).

Approaches have been sought to prevent the accumulation of Cd in plants to reduce Cd content in human diets so as to alleviate health risks associated with exposure to Cd. One of the best cost-effective and efficient approaches is to develop Cd-exclusive genotypes. To breed Cd-exclusive genotype, it is important to find out the potential of Cd accumulation in existing genotypes and their physiological responses to Cd addition. The uptake of Cd varies among plant species, and differences in Cd uptake by roots and its translocation from roots to shoots between genotypes seem to be important determinants of Cd in the harvested products (Athur *et al.,* 2000). Genetic variation in Cd uptake also exists within species, for example, soybean (Bogess *et al.,* 1978), maize (Hinesly *et al.,* 1982) and lettuce (Thomas and Harrison 1991). Few studies have been conducted to determine the effect of Cd on the uptake of plant metal elements, such as Zn, Mn, Cu and Fe. The studies reported to date have provided contradicting results. For instance, the effect of Cd on the uptake of Zn can be synergistic (Turner, 1973; Smith and Brennan, 1983) or antagonistic (Mahler *et al.*, 1982). Jalil *et al.* (1994) found that Cd application decreased the concentration of K, Zn, and Mn in roots and shoots of durum wheat, while the Fe and Cu concentrations in shoots and roots were not affected. Yang *et al.* (1998) reported that addition of Cd to growth medium decreased the growth rate, dry matter yield and as well as the accumulation of Fe, Mn, Cu, Ca and Mg in cabbage, ryegrass, maize and white clover, but increased their P accumulation. Obata and Umebayashi (1997) reported that Ca and Zn did not show any clear tendency with Cd applications in rice, kidney bean, cucumber, pumpkin and maize. Some of these contradictions can be due to concentration dependent interactions of Cd with other

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metals. In bush bean, Fe concentration of plants decreased at low Cd levels, but increased at high Cd levels (Wallace *et al.*, 1977). In contrast, Zn concentration in *Brassica chinensis* increased at low Cd levels but decreased at higher Cd levels (Wong *et al.*, 1984). However, there is little information about the different sensitivity and physiological responses of barley genotypes to Cd toxicity, although barley is a major world crop, ranked as the fourth most important cereals in terms of planting area.

The current work was conducted to determine the genotypic difference of barley in their response to Cd through studying the effect of Cd addition on seedling growth, biomass accumulation, and the uptake and distribution of Cd and other mineral elements in plants.

MATERIALS AND METHODS

This experiment was carried out in 2000 on Huajiachi campus, Zhejiang University, Hangzhou, China. Eleven barley genotypes with different genetic background were used: 7 genotypes of two-rowed and hulled type: Zhenong 12 (tall variety), Zhenong 1 (semi-dwarf), Fenai 2 (semi-dwarf), Aibaiyang, Azhao3 (semi-dwarf), Xiumai 3; and ZAU 3; 1 genotype of two-rowed and naked type: Mimai 114; 1 genotype of four-rowed and naked type: XZ-dingrenqing; 2 genotypes of six-rowed and hulled type: Wumaoliuling (no awn), Xiyin 2.

The seeds were surface sterilized in 0.5% $Ca(OCl)_2$ for 20 min, rinsed in deionized water and germinated in sterilized moist quartz sand at 20°C. When seedlings grew the second leaf (10-day old), they were transplanted to 70-L container containing 60L nutrient solution, which was covered with a wooded-plate with evenly spaced holes and placed in a greenhouse under controlled environment (at 15 ± 3°C). In each hole two seedlings were located. The composition of the basic nutrient solution was (mg L^{-1}): $(NH_4)_2SO_4$ 48.2, $MgSO_4$ 65.9, K_2SO_4 15.9, KNO_3 18.5, $Ca(NO_3)_2$ 59.9, KH_2PO_4 24.8, Fe-citrate 5, $MnCl_24H_2O$ 0.9, $ZnSO_47H_2O$ 0.11, $CuSO_45H_2O$ 0.04, HBO_3 2.9, H_2MoO_4 0.01. The solution pH was adjusted to 5.5±0.1 every other day with NaOH or HCl, as required. At the sixth day after transplanting, Cd as $CdCl_2$ was added to each container to form 3 concentrations: 0 (control), 0.1, and 1 μM. The Cd treatment levels were set up according to soil survey, which showed the normal soil Cd concentration in Zhejiang province, China was about 0.1-0.25μM, while in the contaminated soil was more than 0.5μM (Wu and Zhang). The experiment was laid out as a split-plot design with Cd concentrations as the main plot and genotype as the sub-plot with three replicates. 6 individual plants per genotype per replicate were used. The nutrient solution in the growth container was continuously aerated with pumps and renewed once a week and Cd was also renewed in the exposure solutions.

At the 15 and 25 days after Cd addition, green leaf number was counted, and a chlorophyll meter (Minolta SPAD-502) was used to take SPAD (Soil-Plant Analyses Development) values (chlorophyll meter readings) of the fully expanded leaves (the first from the apex) (Wu *et al.* 1998). Doing the second measurement, plant height and

leaf symptom (according to Cakmak *et al.* 1998) was simultaneously determined. Then seedlings were allowed to grow for additional 1 d in culture solution without Cd, and then harvested, separated into roots and tops (shoots and leaves), dried at 80 °C and weighted. Each sample was digested in a mixture of HNO_3-$HClO_4$ (2:1), and the concentrations of Cd and other mineral elements, such as Cu, Fe, Zn and Mn were determined using a PE-100 Perkin Elmer flame atomic absorption spectrometry.

RESULTS AND DISCUSSION

The most obvious reaction of barley plants to Cd toxicity was characterized by a reduction in SPAD value (Table 1). Although no visual leaf Cd toxicity symptoms of necrotic patches were observed in each of 11 genotypes treated with 0.1 μM Cd, the reduction in SPAD value occurred along with the development of yellow necrotic patches (Table 2) in 1μM Cd treatment. Time of appearance and severity of the Cd toxicity symptoms significantly differed among genotypes ($p<0.01$). Zhenong 1 and Mimai 114 were the genotypes least affected, in terms of SPAD value, leaf number per plant and yellow necrotic patches, whereas, two six-rowed genotypes, Wumaoliuling and Xiyin 2 were the most affected and Cd toxicity symptoms also appeared rapidly and severely.

Table 1. Effect of Cd on leaf number, SPAD value, and plant height of barley expressed as the percentage of control (%)

Treatment	Reduction	SPAD value		Leaf No		Plant height
(μM Cd)	percentage	15[1]	25	15	25	25
0.1	Mean	-5.8*[2]	-25.3**	-2.3	-2.3	-21.1**
	Min	+0.4	-9.4	+5.0	0	-8.1
	Max	-16.8	-41.7	-5.3	-6.7	-36.3
	CV (%)	33.1	46.6	23.1	11.3	6.6
	Between genotypes	**[3]	**	*	*	**
1	Mean	-27.2**	-44.8**	-26.0*	-27.5**	-29.5**
	Min	-11.5	-19.9	-10.0	-16.7	-15.2
	Max	-40.1	-64.7	-53.8	-43.3	-42.3
	CV (%)	42.3	34.4	11.5	44.7	28.1
	Between genotypes	**	**	*	**	**

[1] Days after Cd application.

[2] * and **, Significant at the 0.05 and 0.01 probability levels, respectively, between 0.1, or 1μM Cd treatment and control.

[3]* and **, Significant at the 0.05 and 0.01 probability levels, respectively, between genotypes in 0.1, or 1μM Cd treatment.

Cd toxicity markedly hindered the shoot elongation. Thus, on an average of 11 genotypes, the plant height of 0.1 μM Cd and1μM Cd treatments were 21.1% and 29.5% lower than the control, respectively (Table 1). Moreover, there were also significant differences in plant height among genotypes. Wumaoliuling had the greatest reduction in plant height, while Aibaiyang was the least affected by Cd treatments.

As shown in Table 2, shoot dry weights were more affected than that of root, especially in 1μM Cd treatment. On an average of 11 genotypes, dry weight reduction was 13.2%, 33.5% for shoots and 11.5%, 29.0% for roots in 0.1μM Cd and 1 μM Cd treatments, respectively. There was considerable genotypic variation in reduction of both shoot and root dry weights (Table 2). e.g. in 1 μM Cd treatment, a sharp decline of dry matter production was observed for all 11 genotypes, while Zhenong 1 and Wumaoliuling were the genotypes with the least and the most affected, respectively.

Table 2. Shoot and root dry weight and leaf symptom of different barley genotypes under different Cd treatments

Genotype	Shoot dry weight (mg plant^{-1})			Root dry weight (mg plant^{-1})			Leaf symptom[1]
	Cd treatment (μM)						
	0	0.1	1	0	0.1	1	
Zhenong 12	16.7	16.0 (-4.0)[2]	10.7 (-36.0)	31.7	29.7 (-6.2)	26.7 (-15.6)	3
Xiyin 2	15.6	12.2 (-21.4)	10.6 (-32.1)	37.8	27.7 (-26.8)	25.1 (-33.6)	2
Fenai 2	20.0	17.2 (-13.9)	14.1 (-29.5)	41.1	38.6 (-6.1)	30.7 (-25.4)	3
Zhenong 1	21.1	17.2 (-18.4)	18.1 (-14.5)	40.0	39.3 (-1.9)	33.6 (-16.0)	4
Aibaiyang	22.8	17.8 (-21.9)	17.2 (-24.3)	41.1	34.4 (-16.2)	32.2 (-21.8)	3
XZ-dingrenqing	20.6	12.2 (-40.5)	10.0 (-51.4)	35.6	27.0 (-24.1)	16.7 (-53.1)	3
Mimai 114	17.0	17.9 (+5.2)	12.2 (-28.1)	33.0	33.9 (+2.7)	24.4 (-25.9)	5
Azhao3	14.7	12.8 (-13.2)	9.2 (-37.7)	30.0	26.7 (-11.1)	21.8 (-27.5)	3
Wumaoliuling	14.7	13.5 (-8.1)	8.8 (-40.2)	30.0	21.8 (-27.3)	12.3 (-58.9)	1
Xiumai 3	19.4	19.2 (-1.4	12.1 (-38.0)	41.7	37.5 (-10.0)	30.6 (-26.6)	3
ZAU 3	17.9	17. 8 (-0.9)	11.4 (-36.7)	36.7	36.1 (-1.5)	28.9 (-21.2)	4
Mean	18.2	15.8 (-13.2)	12.1 (-33.5)	36.2	32.1 (-11.5)	25.7 (-29.0)	
$LSD_{.05}$ Between genotypes	3.6	4.2	4.5	6.9	5.8	4.9	
Between Cd treatments		2.3			4.1		

[1] Leaf symptoms of Cd toxicity (necrotic patches on leaf blade) for 1 μ M Cd treatment were measured 25 d after Cd application. 1=very severe, 2=severe, 3=mild, 4=slight, 5=very slight or absent.

[2]Values within bracket represent the relative reduction of Cd-treatments to the control.

The methods used to evaluate heavy metal tolerance were based on root elongation measurements in plants grown with high concentrations of the toxic ion (MacNair, 1993). Root biomass, length and number and rates of root elongation had all been used as indicators of plant tolerance to heavy metal (Baker and Walker, 1989). In this study, root and shoot biomass, shoot elongation, leaf symptoms, and SPAD values were measured, and significant differences in these parameters were found among the 11 barley genotypes. According to these parameters, Mimai 114 and ZAU 3 were the least affected by Cd toxicity and Wumaoliuling was the most affected. SPAD value may be considered the most effective index revealing genotypic difference in their response to Cd-toxicity, while leaf necrotic symptom was observed only in 1 μM Cd treatment, suggesting that SPAD may aid in developing an effective procedure to identify and characterize new metal-tolerant species.

Cd accumulation in roots and shoots increased with increasing Cd concentrations in the medium (Table 3). The Cd concentration was lower in shoots than in roots. In 0.1 and 1μM Cd treatments, the mean root Cd concentration of all 11 genotypes were 9-fold and 10-fold higher than that in the shoot, respectively, indicating that a higher proportion of the Cd taken up by plants remained in the roots during seedling stage. This was in agreement with a number of recent reports, which indicated that metals accumulated more in the roots than in the aboveground parts (Jensen and Adalsteinsson, 1989). Significant differences ($p<0.01$) in Cd concentration and translocation from root to shoot were found among the 11 genotypes in both 0.1μM Cd and 1μM Cd treatments. At 0.1 and 1μM Cd solutions, Mimai 114 and ZAU3 had significantly lower Cd concentrations in both root and shoot than that of other genotypes, while Wumaoliuling and Zhenong 1 had the highest Cd concentrations. However, Zhenong 1 showed high tolerance to Cd toxicity in terms of seedling growth parameters (Table1 and 2). This indicates a type of tissue tolerance but needs further verification. Such tolerance might be due to multiple mechanisms, such as detoxification and sequestration. Ernst *et al.* (1992) reported that metal complexes with phytochelatins, organic acids, and inorganic compounds are responsible for metal tolerance, especially in the case of hyperaccumulator plants, which would prevent Cd from interfering with more sensitive sites of cellular metabolism.

Table 3. Cd concentration and its root to shoot translocation in different barley genotypes grown for 25 d in nutrient solution with different Cd concentrations.

Genotype	Cd concentration (μg g^{-1} DW)						Cd translocation (root/shoot)		
	Shoot			Root					
	Cd treatment (μM)								
	0	0.1	1	0	0.1	1	0	0.1	1
Zhenong 12	0.0197	9.05	12.28	0.0641	61.69	115.72	3.3	6.8	9.4
Xiyin 2	0.0196	10.29	12.26	0.0592	67.96	122.80	3.0	6.6	10.0
Fenai 2	0.0167	9.00	12.18	0.0459	83.69	162.62	2.7	9.3	13.4
Zhenong 1	0.0156	10.17	16.77	0.0418	115.88	174.78	2.7	11.4	10.4
Aibaiyang	0.0136	9.07	11.44	0.0415	98.32	160.80	3.0	10.8	14.1
XZ-dingrenqing	0.0150	9.69	17.72	0.0535	80.94	108.97	3.6	8.4	6.1
Mimai 114	0.0146	7.28	10.87	0.0409	59.26	105.38	2.8	8.1	9.7
Azhao3	0.0195	8.17	14.13	0.0532	74.61	140.16	2.7	9.1	9.9
Wumaoliuling	0.0191	11.01	19.78	0.0618	101.23	169.87	3.2	9.2	8.6
Xiumai 3	0.0178	9.09	12.01	0.0633	74.96	104.34	3.6	8.2	8.7
ZAU 3	0.0170	5.61	11.05	0.0637	61.73	104.49	3.7	11.0	9.5
Mean	0.0171	8.95	13.68	0.0535	80.02	133.63	3.1	9.0	10.0
LSD .05 Between genotypes	ns[1]	0.87	2.03	ns	11.81	15.91	ns	1.9	3.1
LSD .05 Between Cd treatments		2.17			10.37			5.31	

[1] ns, non significant at 95% probability level

In contrast, Mimai 114 showed sharp reduction in plant height and shoot dry weight in 1 μM Cd-treatment (Table 2), although it had the lowest Cd concentration in shoots and roots. This may imply the cost of heavy metal resistance. Some authors (Baker and Walker, 1989; Wilson, 1988) had attempted to determine the costs of

heavy metal resistance, and assumed that it led to slower growth rates and lower biomass production, which has thereby disadvantages compared with non-resistant plants growing on uncontaminated soil. Though this seems to be evident, detailed quantitative studies on a broad scale are still lacking. The question is whether avoidance or tolerance 'costs' more.

There was a significant influence of Cd addition on the uptake and distribution of some microelements (Zn, Mn, Cu and Fe) in plants. In comparison with control, there was a highly significant reduction ($p<0.01$) in Zn concentration over 11 genotypes exposed to 0.1 and 1μM Cd medium by 31.1%, 42.6% in shoots, and 14.9%, 28.0% in roots (Table 4), respectively. Meanwhile, much more Zn was remained in roots.

Table 4. Zn concentration in different barley genotypes grown for 25 d in nutrient solution with different Cd concentrations.

Genotype	Shoot (μg g^{-1} DW)			Root (μg g^{-1} DW)				
	Cd treatment (μM)							
	0	0.1	1	0	0.1		1	
Zhenong 12	132.5	92.0 (-30.5)[1]	81.8 (-38.2)	187.4	135.3	(-27.8)	131.7	(-29.7)
Xiyin 2	168.0	112.2 (-33.2)	80.8 (-51.9)	158.9	113.1	(-28.8)	108.0	(-32.0)
Fenai 2	170.1	121.4 (-28.7)	88.6 (-47.9)	164.9	141.7	(-14.1)	121.2	(-26.5)
Zhenong 1	179.2	131.3 (-26.8)	112.3 (-37.4)	159.0	160.4	(+0.9)	125.2	(-21.2)
Aibaiyang	180.8	121.2 (-32.9)	98.5 (-45.5)	148.6	127.5	(-14.2)	111.4	(-25.1)
XZ-dingrenqing	181.1	117.8 (-35.0)	110.2 (-39.2)	187.8	167.2	(-11.0)	130.8	(-30.4)
Mimai 114	150.1	109.1 (-27.3)	90.1 (-39.9)	171.2	169.1	(-1.2)	117.7	(-31.2)
Azhao3	182.8	117.2 (-35.9)	105.2 (-42.5)	185.4	164.2	(-11.4)	129.7	(-30.1)
Wumaoliuling	147.2	97.1 (-34.0)	75.1 (-49.0)	153.6	113.3	(-26.3)	100.0	(-34.9)
Xiumai 3	145.7	98.8 (-32.2)	89.2 (-38.7)	153.5	133.6	(-13.0)	117.4	(-23.5)
ZAU 3	132.2	98.2 (-25.7)	81.2 (-38.6)	156.3	129.2	(-17.3)	120.2	(-23.1)
Mean	160.9	110.6 (-31.1)	92.11 (-42.6)	166.1	141.3	(-14.9)	119.4	(-28.0)
LSD .05 Between genotypes	39.0	32.6	22.7	29.0	29.3		27.2	
LSD .05 Between Cd treatments		10.6			9.7			

[1] the reduction in Zn concentration of shoot and root of barley for Cd-toxicity expressed as the percentage of control shown in the brackets

Cd addition to nutrient solution also led to the dramatic reduction of Mn and Cu concentrations in the plant tissues and of Fe concentration in shoots (Table 5). In 0.1 and 1 μM Cd treatments, mean Mn concentration over 11 genotypes reduced ($p<0.01$) by 57.7% and 88.0% for shoots, by 48.1% and 81.2% for roots, respectively. Correspondingly, Cu concentration reduced by 6.5% and 77.9% for shoots, by 25.6% and 74.7% for roots, respectively. Although shoot Fe concentration showed the corresponding reduction in Cd treated plants, root Fe concentration had highly significant increase over control. The significant difference also existed for increase of root Fe concentration among 11 genotypes, ranging from 0.8% of Fenai 2 to 63.8% of Azhao 3 in 0.1 μM Cd treatment, and from 11.2% of Xiyin 2 to 296.0% of Azhao 3 in 1 μM Cd treatment.

Table 5. Effect of Cd on mineral concentration of barley expressed as the percentage of control (%).

Treatment	Reduction	Shoot				Root			
(μM Cd)	percentage	Zn	Mn	Cu	Fe	Zn	Mn	Cu	Fe
0.1	Mean	-31.1**[1]	-57.7**	-6.5*	-17.7*	-14.9**	-48.1**	-25.6**	+20.4**
	Min	-26.8	-45.5	+0.3	-6.6	+0.9	-8.7	-1.6	+0.8
	Max	-35.9	-65.2	-22.8	-31.2	-28.8	-68.9	-64.5	+63.8
	CV (%)	11.3	10.6	86.0	51.1	65.6	41.6	71.1	80.9
	Between genotypes	*[2]	**	**	ns[3]	**	**	**	**
1	Mean	-42.6**	-88.0**	-77.9**	-19.1**	-27.9**	-81.2**	-74.7**	+106.3**
	Min	-37.4	-72.3	-55.4	-9.6	-21.2	-75.3	-48.9	+11.2
	Max	-51.9	-92.7	-90.8	-30.8	-34.9	-88.0	-97.8	+296.0
	CV (%)	12.0	7.2	15.6	33.9	15.4	6.7	17.4	116.6
	Between genotypes	**	**	**	ns	*	*	**	**

[1] * and **, Significant at 0.05 and 0.01 probability levels, respectively, between 0.1, or 1μM Cd treatment and control

[2] * and **, Significant at 0.05 and 0.01 probability levels, respectively, between genotypes under 0.1, or 1μM Cd treatment.

[3] ns, non significant at 0.05 probability level.

Therefore, excessive Cd accumulation would affect the rate of uptake and distribution of certain nutrients in the plants, and consequently would be responsible for mineral deficiencies/unbalance and depression of the plant growth. Meanwhile, more addition of Cd (1 μ M) to solution resulted in markedly reduction in Zn and Mn concentrations in shoots and relatively small decreases in root, implying that these elements translocation from roots to shoots was prevented at higher Cd treatments, and may aggravate the mineral deficiency in shoots, which was corroborated to previous reports by Jalil *et al.* (1994) on durum wheat. Rubio *et al.* (1994) also reported that Cd could cause retardation in plant growth by inhibiting nutrient uptake and distribution.

Other reports had concluded that Cd addition decreased the Zn concentration in corn and tomato (Mahler *et al.*, 1982), and sorghum (Mehla *et al.*, 1988). Similar effects of Cd on Mn, Cu, and Fe were observed by Bjerre and Schierup (1985) on oats, Khan and Khan (1983) on tomato and eggplant, and Mahler *et al.* (1982) on lettuce and tomato. There were also contradicting reports on the relationship between Cd and Zn, e.g. Smith and Brennan (1983) reported that uptake of Zn and Cd was synergistic. These conflicting results presumably were due to the differences in the culture methods, species, and conditions such as concentration in medium, growth period and temperature. In this study, Mimai 114, exposed to 0.1μM Cd, showed an slight increase in shoot Zn concentration but a decrease in roots, compared by the control plants (Table 5). The increase in Zn concentration in shoots and its decrease in roots may therefore be attributed to a stimulation of Zn transfer from roots to shoots as reported for tomato and carrot (Turner, 1973) and rice (Dabin *et al.*, 1978). However, at higher Cd concentration (1 μ M), a sharp decrease both in shoot and root was observed.

Significantly negative relationship in mineral concentration between Zn and Cd of shoot (r^2= 0.6774**, n=33) and root (r^2= 0.5187**, n=33) was found, indicating that Zn uptake may inhibit Cd uptake and distribution in plants. It may be assumed that Zn application to Cd contaminated soil would alleviate Cd toxicity in barley. Oliver *et al.* (1994) reported that Cd concentration of wheat grain was reduced with application of Zn in areas of marginal to severe Zn deficiency. They also noted that there was a residual effect of Zn, decreasing the concentration of Cd in wheat grown from 1 to 4 years after application of the Zn fertilizer. McLaughlin *et al.* (1995) found that Cd concentration of potato tubers was negatively correlated with extractable Zn levels in soil. Zn appeared to interfere with the translocation of Cd from roots to young leaves by favoring Cd retention in roots, but at a higher Zn solution, Zn might have further interfered with Cd uptake by the roots. McKenna *et al.* (1993) reported that Zn interfered with the distribution of Cd in lettuce and spinach, but the mechanism involved is to be determined. Therefore, further studies are needed for making clear of interaction between Cd and Zn in their uptake and translocation by plants.

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