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Genotypic Differences in Effect of Cd on Photosynthesis and Chlorophyll Fluorescence of Barley (*Hordeum vulgare* L)

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Soil contamination by heavy metals has become a growing concern worldwide, as some metals are the potential risk for human health when transferred from plant products to the human diet (Grant *et al.,* 1998). Moreover, at high concentrations heavy metals are also toxic to the plants, leading to the growth inhibition and decline in the productivity of crops (Florijn and Beusichem, 1993; Obata and Umebayashi, 1997). Among the heavy metals, Cd is in particular concerned due to its potential toxicity and its relatively high mobility in the soil-plant system.

The presence of excessive amount of Cd in soil commonly elicits many stress symptoms in plants, such as reduction of growth, especially root growth (Weigel and Jäger, 1980), disturbances in mineral nutrition and carbohydrate metabolism (Moya *et al.*, 1993), and may therefore strongly reduce biomass production. The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis (Padmaja *et al*., 1990) and photosynthesis (Bazzaz *et al*., 1975; Baszynski *et al*., 1980). It has been reported that Cd, in particular, inhibited chlorophyll biosynthesis and decreased total chlorophyll content (Padmaja *et al*., 1990). Light and dark reactions of photosynthesis are inhibited by heavy metals at different target sites (Krupa and Baszynski, 1995), photosystem (PS) II being particularly affected (van Assche and Clijsters, 1985; Krupa and Baszynski, 1995). Cadmium is thought to act at PSII on both the oxidizing (donor) and the reducing (acceptor) side. Moreover, PSII reaction centers and PSII electron transport are affected by interaction with Cd, the metal impairing enzyme activity and/or protein structure (van Assche and Clijsters, 1985). In contrast, Haag-Kerwer *et al*. (1999) reported that photosynthesis in *Brassica juncea* was not affected by exposure to 25μM Cd, while transpiration showed a significant decline, in particular, under lower light conditions (≤300μmol photons m^{-2} s^{-1}). There is wide variation in Cd toxicity tolerance among plant species and genotypes within a species (Grant *et al.* 1998). Our previous study found significant difference in the response of seedling growth and nutrient uptake to Cd toxicity among barley genotypes (Wu & Zhang 2002). However, little is known about whether a corresponding difference exists in the response of photosynthesis and chlorophyll fluorescence to Cd toxicity among plant genotypes differing in Cd tolerance.

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The present study was undertaken to investigate the effect of Cd on photosynthesis and chlorophyll fluorescence of four barley genotypes with different Cd tolerance in seedling growth and nutrient uptake, with an effort to quantify the physiological effect of Cd stress on the photosynthetic apparatus of barley plants.

MATERIALS AND METHODS

The experiment was carried out in 2000-2001 growth season at Huajiachi campus, Zhejiang University, Hangzhou, China. Four barley genotypes with different Cd tolerance (Wu and Zhang, 2002) were used: 3 relatively tolerant-genotypes of two-row type: Zhenong 1, ZAU 3 and Mimai 114; 1 relatively sensitive-genotype: Wumaoliuling.

Seeds were surface sterilized in 2% H_2O_2 for 10 min, rinsed with deionized water, and then germinated in sterilized moist quartz sand at 20 ± 1°C. When seedlings grew the second leaf (10-d old), they were selected for uniformity and transplanted to a 6-L container containing 5.5 L nutrient solution, which was covered with a polystyrol-plate with 7 evenly spaced holes and placed in a greenhouse. In each hole two seedlings were located. Eighty-seven days after transplanting (during jointing stage), six plants were left in each container. The composition of the basic nutrient solution was the same as the previous study (Wu and Zhang, 2002). The solution pH was adjusted to 6.5±0.1 every other day with NaOH or HCl, as required. At the sixth day after transplanting, cadmium as $CdCl_2$ was added to each container to form 3 concentrations: 0 (control), 0.1 and 1 μM. From the 40th day after transplanting and thereafter, half of the 1 μM Cd treatments were changed into 5 μM Cd. The experiment was laid out as a split-plot design with Cd concentration as the main plot and genotype as the sub-plot with seven replicates. Fourteen 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.

The measurements were carried out on second fully expanded leaves of plant from the top. Ten days after Cd application, chlorophyll content was determined by the method of Chen (1984) at 10-day intervals. At 70 d after Cd application, 9 plants (3 plants of each replicate) of each treatment 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.

Chlorophyll fluorescence parameters of photosystem II (PSII) were measured with a portable fluorometer (model FMS-2, Hansatech Instruments Ltd., England). The leaves of measuring plants were first adapted for 30 min in total darkness with a Hansatech clip. The unquenchable portion of fluorescence (F_0) was determined by measuring beam (<0.05μmol m^{-2} s^{-1}). The maximal fluorescence (Fm) was determined using a saturating pulse (1200μmol m^{-2} s^{-1}). Actinic light was obtained from a light emitting diode (180 μmol m^{-2} s^{-1}). The variable fluorescence (Fv) was taken from the formula, $Fv=Fm-F_0$. The ratio of variable fluorescence to maximal fluorescence (Fv/Fm) is an indicator of the efficiency of the photosynthetic apparatus (an efficiency of excitation energy capture by open PSII reaction centers), while ΦPSII an actual photochemical efficiency of PSII in the light. In parallel to the fluorescence measurement, photosynthetic parameters, such as net photosynthetic rate (Pn), stomata conductance (Gs) and intracellular CO_2 concentration (Ci), were determined using LCi (leaf chamber analysis) portable photosynthesis system (ADC, Analytical Development Company, England).

RESULTS AND DISCUSSION

As shown in Table 1, root dry weights were more affected than that of shoot, in both 1 and 5μM Cd treatments. On an average of 4 genotypes, dry weight reduction was 11.39%, 20.63% for shoots and 28.54%, 40.59% for roots in 1μM Cd and 5 μM Cd treatments, respectively, when compared with the control (without Cd addition). While a slight increase ($p>0.05$) in 0.1 μM Cd treatment was found. There was a considerable genotype variation in reduction of both shoot and root dry weights (Table 1). Zhenong 1 and Wumaoliuling were the genotypes that were least and the most affected, respectively, consistent with previous findings (Wu and Zhang, 2002). In addition, exposed to 0.1 μM Cd, Wumaoliuling showed a slight but not statistic significant decrease in shoot and root dry weight, whereas other three genotypes showed the slight increase.

Table 1. Effect of Cd on dry weight of barley after 70 days of Cd exposure.

	Shoot dry weight (g per plant)				Root dry weight (g per plant)			
	Cd treatment (μM)							
Genotype	0	0.1	1	5	0	0.1	1	5
Mimai 114	1.54	1.64	1.35	1.15*	0.50	0.52	0.33*	0.34*
ZAU 3	1.37	1.45	1.23	1.11*	0.36	0.38	0.26	0.21*
Zhenong 1	1.51	1.64	1.46	1.39	0.42	0.43	0.38	0.28*
Wumaoliuling	1.52	1.51	1.25	1.11*	0.56	0.54	0.32*	0.25**
Mean	1.49	1.56	1.32	1.18*	0.46	0.47	0.33	0.27*
$LSD_{0.05}$ Between genotypes	ns	0.18	0.21	0.23	0.19	0.15	ns	0.11

* and ** Significance at the 0.05 and 0.01 probability levels, respectively, between 0.1, 1 or 5 μ M Cd treatment and control, and refer to each subset of data within each treatment and can be compared only transversely, and not across lines.
ns, not significant at 0.05 probability level.

The dose- and time-responses of chlorophyll content in leaves are summarized in Table 2. Barley plants exposed to 0.1μM Cd showed a slight but not statistic significant increase ($p>0.05$) in chlorophyll contents relative to control, respectively, whereas exposure to 1μM Cd induced a slight decrease, indicating that Cd toxicity to above-ground part of plants occurs only at the concentration in the medium at least above 0.1 μM. Jalil *et al.* (1994) reported the similar results in wheat. However, Cd is chronic toxic to human even at lower concentration, therefore, it is important to minimize Cd accumulation in plants, particularly in edible parts. Increasing Cd concentration in medium to 5μM induced a sharp decline ($p \leq 0.05$) in these measurements. In addition, it may be seen from Table 2 that the deleterious effect of Cd became more notable with extended exposure of time.

The inhibitory effect of Cd on chlorophyll a was more severe than on chlorophyll b. Thus in plants exposed to 5 μM Cd, averaged over four genotypes and four sampling times, chlorophyll a/b ratio was lowered by 8.8% relative to control. This result was in agreement with Baszynski *et al.* (1980). The decrease in chlorophyll a/b ratio was considered to be a consequence of early senescence brought about by Cd (Barcelo *et al.*, 1988).

On the other hand, in 1μM Cd treatment, significant difference in deleterious effect of Cd on these two parameters was observed among 8 sampling dates. At 20 d after treatment, the detrimental effect became the most intense, being 14.4% in total chlorophyll content, 20.3% in chlorophyll a, and 17.9% in chlorophyll a/b ratio lower than control, respectively. At 50 d after treatment, the reduction of these two measurements was diminished, especially in *cv.* Zhenong 1. This may be attributed to a type of tissue tolerance but needs further verification. Such tolerance might stem from multiple mechanisms, including detoxification and sequestration. Ernst *et al.* (1992) and Tereza *et al.* (2000) reported that metal complexes with phytochelatins, organic acids, and inorganic compounds were responsible for metal tolerance, especially in the case of hyper-accumulator plants.

Table 2. Effects of different Cd treatments on chlorophyll content and a/b ratio in barley.

Treatment (μM Cd)	Days after Cd application							
	10	20	30	40	50	60	70	80
	Total chlorophyll content (mg g^{-1}FW)							
0	1.61	0.97	1.1	1.2	1.2	1.4	1.4	1.1
0.1	1.62(0.6)	0.96(-1.0)	1.1(1.9)	1.2(0.9)	1.3(4.1)	1.4(2.9)	1.5(6.5)	1.1(1.8)
1	1.51(-6.2)	0.83*(-14.4)	1.0*(-9.5)	1.1*(-8.6)	1.2(-4.1)	1.3(-5.1)	1.3(-6.5)	1.0(-8.3)
5					1.1*(-11.5)	1.3(-7.3)	1.3*(-7.9)	0.9*(-13.8)
	Chlorophyll a/b							
0	2.2	1.9	1.6	2.0	2.4	2.2	2.0	2.1
0.1	2.2(0.9)	1.8(-3.1)	1.6(0.7)	2.0(0)	2.4(0.5)	2.2(0.5)	2.1(1.4)	2.1(0.3)
1	2.0(-6.3)	1.6* (-17.9)	1.4* (-8.0)	1.9(-2.5)	2.4(-0.3)	2.2(-0.4)	2.0(-0.6)	2.1(-2.5)
5					2.2* (-9.9)	2.1(-7.2)	1.9(-7.0)	1.9* (-11.1)

Values within bracket represent the relative reduction or increase of Cd-treatments to the control.

* and ** Significance at the 0.05 and 0.01 probability levels, respectively, between 0.1, 1 or 5 μM Cd treatment and control, and refer to each subset of data within each treatment and can be compared only vertically, and not across columns.

Moreover, there was also significant difference in these measurements among genotypes. Wumaoliuling was the most affected genotype with the greatest reduction in chlorophyll content compared to the other genotypes.

Changes in photosynthetic function of barley leaves under Cd stress were determined using chlorophyll fluorescence parameters (Table 3). After 50 and 70 days Cd exposure, a decreasing trend in Fv/Fm ratio and ΦPSII with increasing Cd concentration in the medium was observed, although no statistically significant changes were noted in both 0.1 and 1 μM Cd treatments. Increasing Cd concentration to 5μM, on an average of four genotypes, caused 4.77% reduction in Fv/Fm and 5.43% in ΦPSII at 70 d Cd exposure. Wumaoliuling was the most suppressed, being reduction of 6.11% in Fv/Fm, and 7.53% in ΦPSII, while Zhenong 1 only reduced by 4.13% and 3.99%, respectively. The decline in the Fv/Fm ratio in Cd-stressed plants was primarily due to a decrease in Fm, while Fo value exhibited only minor changes.

Whereas ΦPSII is an indicator of actual photochemical efficiency of PSII in the light, the ratio of Fv/Fm is often used as a stress indicator, describing the potential yield of the photochemical reaction. The decrease of these two parameters for the plants in 5 μM Cd treatment suggests a fall in efficiency of the photochemical reduction of Q_A, the primary quinone acceptor of PSII. The fluorescence rise from Fo to Fm is considered to reflect reduction of the primary electron acceptor of PSII, and in the consequence, a Cd-induced inhibition of the Fv (Fm-Fo) may indicate an inhibitory site on the photo-oxidizing side of PSII. The significant decrease of quantum yield in the primary photochemistry process of PSII in 5 μM Cd treatment is especially responsible for the reduced quantum yield of O_2 evolution (Ouzounidou *et al.* 1993). The decrease in plant growth under Cd-stress can therefore be related to its effect on photosynthesis. However, several *in vitro* studies indicated that inhibition of photosynthesis can not entirely be attributed to the direct interference of the heavy metal with photo-reactions since CO_2 fixation was inhibited by Cd without any perceptible effect on photochemical reactions in isolated protoplasts and chloroplasts (Ascencio and Cedeno-Maldonado, 1979; Weigel, 1985). This apparently implies that Cd limits the rate of photosynthesis. In the case of 0.1 or 1 μM Cd-treated plants, the minor reductions in the Fv/Fm ratio and ΦPSII indicate no apparent change in the rate of electron transport from PS II to the primary electron acceptors.

Earlier investigations demonstrated a notable reduction in the rate of photosynthesis by Cd in plants (Baszynski *et al.*, 1980; Sawhney *et al.*, 1990). Our results were not consistent with these observations. As shown in Table 4, the plants exposed to 0.1μM Cd showed a slight but not significant increase in net photosynthetic rate relative to control, while exposure to 1μM Cd induced a slight decrease. Under 5 μM Cd, the mean net photosynthetic rate of 4 genotypes was reduced by 11.5% and 11.8% at the 50 and 70 d after Cd treatment, respectively, compared with control. It was also found that the extent of the negative effect of heavy metals on the photosynthetic apparatus depends on genotype and growth stage. For instance, the reduction in 1μM Cd treated plants ranged from 2.4% of Zhenong 1 to 27.3% of Wumaoliuling at tillering stage (30 d after Cd application,), and from 0.3% of ZAU 3 to 7.6% of Wumaoliuling at stem elongation stage (70d after Cd application,), respectively. Similarly, in 5 μM Cd treatment, Cd exerted slight effect on net photosynthetic rate of Zhenong 1, but significantly reduced net photosynthetic rate in Wumaoliuling by 20.3% relative to control.

Table 3. Effect of Cd treatments on chlorophyll fluorescence parameters in 4 barley genotypes.

	Cd treatment (μM)							
	50 d after Cd application				70 d after Cd application			
Genotype	0	0.1	1	5	0	0.1	1	5
	Fo							
Mimai 114	115	114	113	116	128	128	122	130
ZAU 3	117	116	113	116	129	128	127	131
Zhenong 1	116	114	113	114	129	119	131	127
Wumaoliuling	115	114	114	119	130	130	130	137
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	ns	ns
	Fm							
Mimai 114	892	836	826	819*	863	859	828	812
ZAU 3	843	858	828	814	886	865	855	813*
Zhenong 1	889	846	835	828	871	860	830	815
Wumaoliuling	896	827	825	820*	872	829	821	795*
$LSD_{0.05}$	23.3	20.1	ns	11.5	20.1	20.3	25.3	15.2
	Fv/Fm							
Mimai 114	0.870	0.868	0.856	0.851	0.866	0.851	0.842	0.825*
ZAU 3	0.871	0.865	0.861	0.856	0.871	0.859	0.854	0.835
Zhenong 1	0.872	0.863	0.855	0.861	0.872	0.863	0.855	0.836
Wumaoliuling	0.869	0.861	0.850	0.831*	0.868	0.859	0.850	0.815*
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	ns	ns
	ΦPS II							
Mimai 114	0.699	0.697	0.694	0.689	0.791	0.786	0.785	0.743*
ZAU 3	0.686	0.684	0.682	0.679	0.778	0.773	0.768	0.746
Zhenong 1	0.698	0.699	0.691	0.692	0.777	0.774	0.769	0.746
Wumaoliuling	0.681	0.680	0.659	0.659	0.784	0.780	0.772	0.725**
$LSD_{0.05}$	ns	ns	ns	0.025	ns	ns	ns	0.018

* and ** Significance at the 0.05 and 0.01 probability levels, respectively, between 0.1, 1 or 5 μ M Cd treatment and control, and refer to each subset of data within each treatment and can be compared only transversely, and not across lines.

ns, not significant at 0.05 probability level.

Table 4. Effects of Cd treatment on photosynthesis of 4 barley genotypes.

	30 d after Cd application			50 d after Cd application				70 d after Cd application			
	Cd treatment (μM)										
Genotype	0	0.1	1	0	0.1	1	5	0	0.1	1	5
	Net photosynthetic rate (Pn) (CO_2 μmol m^{-2} s^{-1})										
Mimai 114	19.8	20.0	17.8	19.1	20.2	17.2	17.5	33.7	34.7	33.0	30.0
ZAU 3	18.6	19.1	17.2	20.5	20.3	19.2	17.5*	34.5	35.4	34.4	30.0
Zhenong 1	18.6	18.9	18.1	18.0	19.3	17.6	17.1	34.1	34.3	33.7	33.2
Wumaoliuling	22.1	21.9	16.1*	18.1	17.3	16.0	14.8*	34.4	31.6	31.8	27.4
$LSD_{0.05}$	2.5	2.7	1.8	ns	ns	ns	2.0	ns	ns	3.1	3.5
	Stomata conductance (Gs) (mol m^{-2} s^{-1})										
Mimai 114	1.3	1.2	1.1	1.9	1.8	1.7	1.5*	1.8	1.8	1.7	1.6
ZAU 3	1.0	0.9	0.9	2.1	2.0	1.8	1.7*	2.1	2.0	2.0	1.8*
Zhenong 1	1.3	1.3	1.1	2.0	2.1	1.9	1.8	2.3	2.4	2.1	1.9*
Wumaoliuling	1.2	1.1	1.0*	1.9	1.7	1.8	1.7	2.0	1.9	1.8	1.8
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	ns	0.4	ns	ns	ns
	Intracellular CO_2 concentration (Ci) (μl L^{-1})										
Mimai 114	190	195	207	213	192	238	238	193	175	189	216
ZAU 3	186	181	203	212	203	225	230	192	184	196	205
Zhenong 1	197	188	195	198	187	226	226*	180	170	197	206*
Wumaoliuling	170	175	205*	215	234	271*	280*	195	200	225*	233*
$LSD_{0.05}$	ns	ns	ns	ns	ns	41.5	41.1	ns	ns	33.2	22.2

* and ** Significance at the 0.05 and 0.01 probability levels, respectively, between 0.1, 1 or 5 μ M Cd treatment and control, and refer to each subset of data within each treatment and can be compared only transversely, and not across lines.

ns, not significant at 0.05 probability level.

Sheoran *et al.* (1990a) showed that Cd concentrations of 56 and 112 mg L^{-1} inhibited net photosynthesis to about 50% at the early stage of pigeon pea (30-day-old plants) and did not exert any significant effect on that process at the later stages (70-day-old plants). Our data on net photosynthetic rate of *cv.* Zhenong 1 exposed to 1 and 5 μM Cd confirmed this observation, however, the case was not true for Wumaoliuling. The observed tendency of decreasing in the photosynthetic rate of Cd-treated plants of *cv.* Wumaoliuling throughout the whole growth period could be partly attributed to reduced chlorophyll content (Table 1) or partly to reduced Fv/Fm and ΦPSII (Table 3). Vassilev *et al.* (1997) reported that the negative effect of Cd on photosynthesis was connected with the inhibition of primary carbon metabolism. However, the relatively weaker Cd effect on net photosynthetic rate of Zhenong 1 at later stage could probably be due to an adaptive change in metabolism, or metal tolerance mechanism occurred in barley plants.

On the other hand, the reduction in biomass of 1 and 5 μM Cd-treated plants was more severe than in net photosynthetic rate. For example, on an average of 4 genotypes shoot dry weight reduced by 11.39% and 20.63% at 70 d Cd exposure in 1 and 5 μM Cd, respectively, although its corresponding net photosynthetic rate reduction was 2.8% and 11.8%. This may imply the cost of heavy metal resistance. Some researchers (Baker and Walker 1989; Wolfgang and Helmuth 1993) had attempted to determine the cost of heavy metal resistance, and assumed that it led to slower growth rate and lower biomass production, which is thereby disadvantage compared with non-resistant plants growing on uncontaminated soil. Though this seems to be evident, detailed quantitative study is still lacking. Furthermore, it also indicates that the detrimental effect of Cd on biomass is the result of complicated physiological and morphological stress-response. In this study average photosynthetic leaf area per plant over 4 genotypes declined by 8.3% and 21.6% after 70 days Cd exposure to1 and 5 μM relative to control.

Concerning to the intracellular CO_2 concentration (Ci), in 0.1 μM Cd treatment, mean Ci of 4 genotypes reduced slightly ($p>0.05$) by 0.5%, 2.6%, and 4.2% in 30, 50 and 70 days Cd exposure, respectively. This may be due to the slight increase in net photosynthetic rate. Increasing Cd concentration to 5 μM, intracellular CO_2 was significantly increased for both *cv.* Wumaoliuling and Zhenong 1, even though Zhenong 1 was the least affected genotype in terms of net photosynthetic rate.

As to stomatal conductance (Gs), addition of 1 or 5 μM Cd in culture solution caused different inhibiting effect on 4 genotypes; however, the results obtained were not consistent to the change of net photosynthetic rate. The deleterious effect did not become pronounced with extended exposure to concentrated Cd. It may be suggested that more than 1 μM Cd would induce closing of stomata and result in the reduction in Gs, but was not the direct or solely reason for the reduced net photosynthetic rate. Sheoran *et al.* (1990b) reported that reduction of CO_2-exchange rate could not be attributed to any single factor and appeared to be due to the combined effects on stomatal conductance, chlorophyll content and on the functioning of photosynthetic apparatus. Malik *et al.* (1992) showed that reduced CO_2 fixation in Cd-treated wheat seedlings was not accompanied by decreased stomatal conductance. In contrast, Barcelo and Poschenriender (1990) reported that the disturbed water relation to plants was one of the main reasons for the heavy metal phytotoxicity. However, there is little information about changes of photosynthesis response to Cd during ontogenesis in plants grown in Cd-contaminated soil.

The present study indicates that the negative effect of Cd on net photosynthetic rate is due to a complex of physiological disturbances, including the inhibition of chlorophyll biosynthesis, reduction in Fv/Fm and ΦPSII and disordered stomata behavior. The decline in the Fv/Fm ratio in Cd-stressed plants was primarily due to a decrease in Fm or attributed to decreased Fv (Fv=Fm-Fo). There was a noticeable difference among barley genotypes in the effect of Cd on photosynthesis and chlorophyll fluorescence parameters and the difference basically conformed to the growth inhibition of varying magnitude. In addition, it was interesting to note that Cd applied at a concentration of 0.1μM in the medium caused slight increases in chlorophyll content and biomass, suggesting the potentially positive impact of Cd on plant growth at low concentration.

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