BRIEF COMMUNICATION

Effect of Cd on growth, photosynthetic gas exchange, and chlorophyll fluorescence of wild and Cd-sensitive mutant rice

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Abstract

Growth, photosynthetic gas exchange, and chlorophyll fluorescence characteristics were investigated in wild type (WT) and Cd-sensitive mutant rice (*Oryza sativa* L.) plants using 50 μM Cd treatment for 12 d followed by a 3-d recovery. Under Cd stress, net dry mass and pigment contents were significantly lower in the mutant plants than in the WT. The mutant had lower net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) than WT rice, however, it had higher intercellular CO_2 concentration (C_i), indicating that non-stomatal factors accounted for the inhibition of P_N. Maximal photochemical efficiency of photosystem 2 (F_v/F_m), effective quantum yield of PS2 (Φ_{PS2}), and photochemical quenching (q_P) decreased much in the mutant under Cd stress. Cd content in roots and leaves of the mutant was significantly higher than those in the WT. Hence Cd toxicity was associated with the marked increases in Cd contents of plant tissue. After the recovery for 3 d, the WT rice had higher capacity to recover from Cd injury than the mutant.

Additonal key words: chlorophyll; growth; intercellular CO_2 concentration; *Oryza*; recovery; stomatal conductance; transpiration rate.

Photosynthetic organisms are highly sensitive to Cd (Clijsters and Van Assche 1985, Das *et al*. 1997, Prasad and Zeeshan 2005). Several studies have been focused on the inhibition of photosynthesis, yet the mechanisms of Cd toxic effect on photosynthetic processes are not yet clear (Clijsters and Van Assche 1985, Krupa and Baszyński 1995). Photosystem (PS) 1 activity is only slightly affected by Cd (Clijsters and Van Assche 1985, Siedlecka and Krupa 1996, Pál *et al*. 2006), but Cd may alter photosystem 2 (PS2) activity (Atal *et al*. 1991, Siedlecka and Krupa 1996, Prasad and Zeeshan 2005, Pál *et al*. 2006). Photosynthetic pigments, stomata, and Calvin-cycle enzymes have been identified as primary targets of Cd (Siedlecka and Krupa 1996, Prasad and Strzałka 1999, Burzyński and Kłobus 2004, Burzyński and Żurek 2007).

Stress responses are determined by stress type and intensity (Ernst 1996) and plant species and tissues being affected (Sanita di Toppi and Gabbrielli 1999, Chen *et al*. 2007). Differences in stress tolerance among plant species and genotypes within a species may be associated intrinsically with the genetic basis. Hence, studies on mutant genotypes with the same genetic background help understand the tolerance/sensitive mechanism of a plant under Cd-induced stress. Howden and Cobbett (1992) obtained Cd-sensitive mutants of *Arabidopsis*, *cad1* and *cad2*. The important role of phytochelatin (PC) expression for heavy metal tolerance is well characterized by mutant studies (Howden and Cobbett 1992, Howden *et al*. 1995).

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Abbreviations: C_i – intercellular CO_2 concentration; Car – carotenoid; Chl – chlorophyll; DM – dry mass; E – transpiration rate; FM – fresh mass; F_m – maximal Chl fluorescence yield; F_0 – minimum fluorescence yield; F_v – variable Chl fluorescence; F_v/F_m – maximal photochemical efficiency; g_s – stomatal conductance to water vapour; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; q_N – non-photochemical quenching; q_P – photochemical quenching; PS2 – photosystem 2; RGR – relative growth rate; WT – wild type; Φ_c – apparent quantum yield; Φ_{PS2} – effective quantum yield of PS2.
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In a previous study we found that Cd-sensitive mutant rice had greater Cd influx into the root, accumulated more Cd in the shoot, and expressed lower catalase (CAT) activities than the wild type (WT) seedlings (Chen *et al*. 2007, He *et al*. 2007). However, little is known about their photochemical responses to Cd. This is why we compared the Cd-sensitive mutants with the WT rice plants for their growth, pigment contents, gas exchange, chlorophyll (Chl) fluorescence parameters, and Cd content after Cd treatment and recovery to have a better understanding of Cd toxic effects on rice with the same genetic background.

The seeds of WT rice (*Oryza sativa* L. cv. Zhonghua 11) and the Cd-sensitive rice mutant, which was obtained from the rice mutant populations constructed with T-DNA (Ac/Ds) insertion mediated by *Agrobacterium* using *japonica* rice as a receptor and identified as a homozygote after four generations of self-hybridization (Zhu *et al.* 2003, Chen *et al.* 2007, He *et al.* 2007) were surface sterilized in 0.5 % sodium hypochlorite for 20 min, rinsed, and germinated in the dark on moistened filter paper at 30 °C for 2 d. After germinating on a plastic screen floating on distilled water at 28 °C for 4 d, uniformly germinated seedlings were transferred to black polyethylene barrels containing 6 000 cm^3 of complete nutrient solution (He *et al*. 2007). Seedlings were grown in a growth chamber with a photon flux density of 500 μmol s^{-1} m^{-2}, average relative humidity of 65 %, and day/night temperatures of 32/27 °C (14/10 h). During the growth period, the solution in each pot was renewed every 6 d. Then, 35-d-old seedlings of the mutant and WT were exposed to 50 μM Cd solution (applied as $CdCl_2$) while those without Cd served as controls. Each treatment was carried out in triplicate. The nutrient solution was renewed every 4 d. Gas exchange, Chl fluorescence, and pigment content were measured after exposure to Cd treatment for 12 d. Then half of plants were harvested and dry mass (DM) and Cd content were measured. Other plants were placed in the fresh nutrition without Cd for another 3 d. The contents of Chl and carotenoids (Car) were measured according to Lichtenthaler and Wellburn (1983). At harvest, plant height was measured and roots were soaked in 15 mM Na_2EDTA for 20 min to remove metal ions adhering to the root surface. Harvested plants were separated into leaves, stems, and roots and dried for at least 72 h at 65 °C until constant mass was reached. DM values were recorded and relative growth rate (RGR) was calculated (Wickens and Cheeseman 1988). Cd contents in leaves, stems, and roots of seedlings was determined by a furnace atomic absorption spectrometry (*SolAAR-M6*, *Thermo Electron,* USA) after wet-ashing of DM in a $HNO_3/HClO_4$ mixture (4 : 1, v/v).

Gas exchange was measured *in situ* using a portable infrared gas analyzer (*LI-6400 LI-COR*, Lincoln, USA) on the second fully developed leaf during 10:00 to 14:30 (Beijing time) at the end of the Cd treatment and recovery. In the measurements, CO_2 concentration was controlled at 385 μmol(CO_2) mol^{-1} with *LI-COR* CO_2 injection system, and a saturating photosynthetic photon flux density (PPFD) of 1 200 μmol m^{-2} s^{-1} from a *LI-COR* LED irradiation source was supplied. Air temperature of leaf chamber was maintained at about 30 ºC. Before recording data, the measured leaves were kept in the leaf chamber for 2 min to reach a steady state of photosynthesis. In apparent quantum yield of carbon assimilation (Φ_c) measurement, CO_2 concentration was kept at 385 μmol(CO_2) mol^{-1}, and PPFD was set at 150, 120, 90, 60, and 30 μmol m^{-2} s^{-1} in turn.

Chl fluorescence was measured on fully-expanded leaves near those used for photosynthesis measurement with an integrating fluorescence fluorometer (*LI-6400–40* leaf chamber fluorometer, *LI-COR*, USA) at room temperature of about 28 ºC. F_0 (minimal fluorescence), F_m (maximal fluorescence), F_v (variable fluorescence), and F_v/F_m (maximal photochemical efficiency of PS2) were measured shortly after keeping the leaves in dark for 30 min. A red irradiation of 7 000 μmol m^{-2} s^{-1} was applied for measurements. Fluorescence parameters, effective quantum yield of PS2 (Φ_{PS2}), photochemical (q_P) and non-photochemical quenching (q_N) were measured on light-adapted leaves using the equations of Genty *et al*. (1989). Statistical significance of the means was compared by Duncan's New Multiple Range Test at the 5 % probability level using *SPSS 10.0* software.

After 12-d Cd treatment, visible symptoms of injury appeared on the old leaves characterized by chlorotic and/or necrotic areas on the margins of both genotypes. Both the mutant and WT plants showed a reduction in net DM and plant height (Table 1). In comparison with WT plants, the growth of the mutant was strikingly inhibited, resulting in dwarf plants with lower DM and RGR (Table 1). However, this inhibition of DM seemed to recover better in the WT than the mutant after 3 d of recovery from stress. Atal *et al*. (1991) and Moya *et al.* (1993) also found a reduction of both shoot and root length and DM in wheat and rice plants treated with Cd. The reduction in growth could be a consequence of Cd interference with a number of metabolic processes associated with normal development, such as cell division of meristematic cells (Prasad and Strzalka 1999, Dalla Vecchia *et al*. 2005), photosynthesis and translocation of photosynthetic products (Krupa and Baszyński 1995, Prasad and Strzalka 1999, Prasad and Zeeshan 2005, Burzyński and Żurek 2007).

Similar to growth, contents of Chl *a,* Chl *b*, and Car were reduced by Cd (Table 1). The inhibitory effect of Cd on Chl content was more pronounced than on Car. In contrast, Chl *a* content decreased markedly. These results were consistent with previous reports (Vajpayee *et al*. 2001, Prasad and Zeeshan 2005, Burzyński and Żurek 2007). Cd could inhibit Chl biosynthesis by means of a reaction with the thiol groups of the enzymes of 5-aminolevulinic acid synthesis and protochlorophyllide

Table 1. Plant height, net increase of dry mass (DM), relative growth rate (RGR), contents of photosynthetic pigments, and photosynthetic and chlorophyll fluorescence parameters of Cd-sensitive mutant and wild type (WT) seedling after 12-d 50 μM Cd treatment and 3-d recovery. Means ± SE, $n \geq 3$, means in a row followed by a different letter are significantly different ($p<0.05$) according to Duncan test.

Parameter		Control	50 μM Cd	Recovery
Plant height [cm]	Mutant	18.1±1.2 a	11.8±0.7 c	12.2±0.8 c
	WT	17.8±1.1 a	14.4±0.8 b	15.7±0.9 b
DM [g plant^{-1}]	Mutant	0.760±0.053 a	0.391±0.028 d	0.425±0.032 d
	WT	0.747±0.046 a	0.533±0.036 c	0.662±0.046 b
RGR [g d^{-1}]	Mutant	0.096±0.004 a	0.054±0.003 d	0.051±0.004 d
	WT	0.094±0.005 a	0.075±0.004 c	0.086±0.004 b
Chl [g kg^{-1}(FM)]	Mutant	3.59±0.17 a	2.15±0.12 c	2.22±0.14 c
	WT	3.59±0.22 a	2.80±0.15 b	3.31±0.17 a
Chl *a* [g kg^{-1}(FM)]	Mutant	2.78±0.16 a	1.55±0.11 c	1.61±0.10 c
	WT	2.78±0.15 a	2.11±0.13 b	2.57±0.14 a
Chl *b* [g kg^{-1}(FM)]	Mutant	0.803±0.041 a	0.621±0.025 c	0.610±0.021c
	WT	0.812±0.044 a	0.682±0.029 b	0.744±0.036ab
Car [g kg^{-1}(FM)]	Mutant	0.831±0.044 a	0.601±0.021 c	0.631±0.032 c
	WT	0.845±0.046 a	0.714±0.035 b	0.797±0.041 a
P_N [μmol m^{-2} s^{-1}]	Mutant	21.60±1.20 a	9.22±0.46 d	10.10±0.52 d
	WT	21.30±0.80 a	14.00±0.90 c	17.70±1.10 b
g_s [mol m^{-2} s^{-1}]	Mutant	0.42±0.03 a	0.21±0.02 c	0.28±0.02 b
	WT	0.41±0.03 a	0.29±0.02 b	0.38±0.02 a
C_i [μmol mol^{-1}]	Mutant	259±10 c	345±12 a	331±13 a
	WT	262±8 c	305±10 b	278±10 c
E [mmol m^{-2} s^{-1}]	Mutant	5.23±0.21 a	3.62±0.25 c	3.71±0.23 c
	WT	5.02±0.28 ab	3.95±0.22 c	4.75±0.29 b
Φ_c	Mutant	0.045±0.003 a	0.028±0.002 c	0.031±0.003 bc
	WT	0.046±0.003 a	0.035±0.002 b	0.041±0.003 a
F_0	Mutant	95.00±6.64 b	110.7±7.2 a	100.4±8.6 ab
	WT	92.30±5.71 b	95.2±5.6 b	91.3±5.1 b
F_m	Mutant	524±24 a	389±13 c	416±14 c
	WT	518±20 a	461±17 b	496±21 a
F_v/F_m	Mutant	0.819±0.019 ab	0.716±0.014 e	0.759±0.021 d
	WT	0.822±0.016 a	0.794±0.013 c	0.816±0.023 b
Φ_{PS2}	Mutant	0.494±0.017 a	0.289±0.014 d	0.312±0.016 d
	WT	0.489±0.013 ab	0.412±0.013 c	0.464±0.013 b
q_P	Mutant	0.837±0.021 a	0.412±0.033 d	0.466±0.036 d
	WT	0.840±0.028 a	0.655±0.026 c	0.761±0.033 b
q_N	Mutant	0.424±0.017 d	0.627±0.021 a	0.595±0.019 a
	WT	0.432±0.015 d	0.515±0.022 b	0.461±0.021 cd

reductase complex (Vajpayee *et al*. 2001). Moreover, a possible substitution of the central magnesium ion of the Chl molecule by heavy metal atoms (Hg, Cd, Cu, Ni, Zn, or Pb) in plants living in an environment containing heavy metal ions has been observed (Küpper *et al*. 1996). Highly significant differences in Chl *a* and Car were recorded between the WT and mutant. After 12 d of exposure, total Chl content in the leaves of the mutant was 23.2 % lower than in WT. Further, Chl and Car contents in WT after recovery were significantly higher than those after Cd treatment. However, they were not different in the mutant.

Cd stress strongly reduced the photosynthetic performance in mutant leaves: net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) were decreased by 57.3, 50.0**,** and 30.8 %, respectively, in comparison to control. However, the internal CO_2 concentrations (C_i) were significantly higher (Table 1), suggesting that not stomatal limitation but decrease of Chl content and F_v/F_m contributed to the decrease of P_N. This is consistent with works of Moya (1993) and Burzyński and Kłobus (2004). Moreover, P_N and g_s of the mutant were more inhibited: they were 34.1 and 27.6 % lower than those of WT. Φ_c in the mutant rice was significantly lower than that in WT. After 3 d of recovery, P_N, g_s, C_i, and Φ_c in the WT plants were similar to those in the control. However, the recovery of photosynthesis in the mutant was not significant (Table 1).

Table 2. Cd contents [mg kg^{-1}(DM)] of root, stem, and leaf of Cd-sensitive mutant and wild type (WT) seedling after 12-d Cd treatment and 3-d recovery. Means ± SE, n = 3, means in a column followed by a different letter are significantly different (p<0.05) according to Duncan test.

Parameters		Control	50 μM Cd	Recovery
Root	Mutant	2.36±0.18 d	1036.00±65.00 a	995.00±47.00 a
	WT	2.28±0.21 d	788.00±42.00 b	646.00±37.00 c
Stem	Mutant	0.69±0.05 c	126.00±6.70 a	120.00±5.90 ab
	WT	0.61±0.04 c	106.00±5.80 b	88.00±4.40 c
Leaf	Mutant	0.25±0.02 d	32.20±2.30 a	29.90±2.10 a
	WT	0.23±0.01 d	22.60±1.60 b	17.10±1.50 c

The ratio of F_v/F_m is always used as a stress indicator, representing the maximum quantum yield of PS2 photochemistry (Maxwell and Johnson 2000, Linger *et al*. 2005). In the present experiment, the F_v/F_m ratios of the mutant and WT leaves before Cd stress were very similar (Table 1). After 12 d of Cd exposure, the F_v/F_m of mutant leaves was significant lower than that in WT. After 3 d of restoration, F_v/F_m of the WT leaves was increased near to initial value, while F_v/F_m of the mutant was still significantly lower than that of the controls. This is consistent with the finding in wild soybean species (Kao *et al*. 2003) and barley (Wu *et al*. 2003). In the mutant, F_0 values slightly increased and a decrease in F_v/F_m could therefore be mainly ascribed to a decrease in F_m.

Φ_{PS2} showed similar changes to F_v/F_m (Table 1). Similar result was obtained for plants treated with excess heavy metals (Vassilev and Manolov 1999, Burzyński and Klobus 2004). Φ_{PS2} in the mutant leaves decreased more markedly during the stress and restored more slowly than that of the WT leaves.

Quenching analysis also revealed a significant decrease in q_P and increase in q_N after 12-d Cd stress, and a near complete recovery in WT 3 d after stress withdrawal (Table 1), while Cd stressed mutant plants showed a much lower q_P and higher q_N compared with WT. These results were consistent with other studies (Vassilev and Manolov 1999, Otero *et al*. 2006).

The Cd content in roots, stems, and leaves of rice plants increased significantly after exposure to Cd and the roots always accumulated the highest Cd amount (Table 2), which reached a maximum of 1 036 mg(Cd) kg^{-1}(DM) in plants after 12 d. Compared to the WT plants, the mutant plants revealed a better ability for both Cd uptake and accumulation in roots and leaves (1.31 and 1.42 times more Cd, respectively). Cd contents of roots, stems, and leaves in the WT were reduced significantly after recovery. However, the mutant had no significant difference in Cd content after 3 d recovery. The higher content of Cd in leaves may cause higher inhibition of photosynthesis for the mutant.

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