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Rapid screening of cadmium tolerant rice (*Oryza sativa* L.) through morpho-physiological markers

Md Azizul Bari[1], Ahmad Humayan Kabir[2*], Md Abu Reza[3]

[1]Institute of Biological Sciences, University of Rajshahi, Rajshahi 6205, Bangladesh
[2]Molecular Plant Physiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh
[3]Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi 6205, Bangladesh
*Correspondence: ahmad.kabir@ru.ac.bd

Abstract

Cadmium (Cd) is a toxic metal for plant growth and development. In this study, sixteen rice lines were screened for Cd tolerance under different concentration of $CdSO_4$ at seedling stage using a rapid hydroponic technique. Among the studied lines, Sonarbangla was categorized as tolerant; BRRI 54 was graded as moderately tolerant and 13 as susceptible based on the scoring in response to different levels of Cd stresses. Results revealed that Sonarbangla had less damage in root and shoot development along with chlorophyll synthesis ratio at seedling stage thus providing a clue about Cd tolerance potential of a genotype. Further, comparative studies in these rice genotypes under hydroponic conditions showed that Cd sensitivity in rice is primarily related to a decrease in root and shoot components. Our findings reveal Sonarbangla as Cd tolerant variety that may boost up the production of rice in Cd-contaminated area and development of Cd tolerant rice trough breeding and transgenic approaches.

Keywords: Rice, cadmium stress, hydroponic culture, cadmium tolerance, screening.

INTRODUCTION

Agricultural soils are slight to moderately contaminate with toxic heavy metals that restrict the crop plants to reach their full genetic potential and cause significant loss by reducing the crop productivity (Yadav 2010). In recent years, heavy-metal pollution of the soil has been causing even more significant problems. Heavy metals accumulated in plants may, either directly or indirectly, find their way into animals and human beings (Nagaveni et al. 2011). Cadmium (Cd) is a non-essential trace element for plants. Cd is a potential and persistent environmental contaminant, causing severe damage to all living organisms, including humans and plants. Cd has been ranked 7th among the top 20 toxins and considered human carcinogen (Yang et al. 2004, Gill & Tuteja 2011). It is a divalent heavy metal cation,which has substantial toxicity at low concentrations (Schützendübel et al. 2001).

Cadmium (Cd) is widely released into the soil, air, and water mainly by effluent from industrial sources, mining burning, leakage waste, sewage sludge and phosphate fertilization (Pinto et al. 2004). Cd can be quickly taken up by plant roots, and transported to the aerial parts where it significantly impedes vital cellular processes, including respiration and CO_2 fixation (Gill & Tuteja 2011, Sandalio et al. 2001). Cd damages the photosynthetic apparatus (Krupa 1988, Siedlecka & Baszynski 1993), decreases chlorophyll content (Stobart et al. 1985, Larsson, Bordman & Asp 1998), inhibits the stomatal opening (Barcelo & Poschenrieder 1990), decreases carbon assimilation and finally leads to wilting and plant death (Hsu and Kao 2003, Rodriguez-Serrano et al. 2009). Chlorosis, necrosis, epinasty, stunted growth, cell death and disturbance in mineral homeostasis are the typical consequences of Cd toxicity in plants (Sandalio et al. 2001, Ortega-Villasante et al. 2005, Nazar et al. 2012).

Effects of Cd stress on plant growth and development were reported in several studies (Hegedus et al. 2001, Prasad et al. 2001, Shah et al. 2001). Many reports suggest importance to consider chronic effects of Cd exposure through foods (Jarup and Akesson 2009). In humans, Cd exposure has been associated with cancer of the prostate, lungs, and testes, kidney tubule damages, rhinitis, emphysema, osteomalacia, and bone fractures (Bertin and Averbeck 2006, Nawrot et al. 2006).

The contamination of food crops by Cd is a major concern in food production as it can reduce crop yields and threaten human health. Cd toxicity has been extensively studied in various food crops including rice (Ge et al. 2009). In many South and East Asian countries, including Bangladesh, India, Japan, Indonesia and Thailand, Cd accumulation in rice and its subsequent transfer to the human food chain is a major environmental issue (Bolan et al. 2013). In Bangladesh, rice cultivating lands adjacent to the industrial establishments are highly contaminated with Cd that was found to be between 134 and 156 mg Cd Kg^{-1} of soil (Naser et al. 2009). Thus, preventing Cd uptake in rice plants grown in Cd-contaminated soils has become an urgent task to ensure food safety. Rice, an important staple food for nearly a half of the world's population, is a major source of Cd intake (Cheng et al. 2006, Watanabe et al. 2004). Rice grains contaminated with Cd represent a major risk to the health of more than half of the world's population, which depends on rice as a primary staple. Therefore, it is indeed a priority work to determine the relative Cd efficiency of staple crops like rice. Among the different screening methods, hydroponic culture has often been used for screening for tolerance to mineral deficiency and toxicity.

Despite the importance of Cd-tolerant crops, no efforts have been made to screen for Cd-tolerant genotypes among Bangladeshi rice lines. Thus, the present study was aimed at screening different rice genotypes mainly cultivated in Bangladesh tolerant to Cd stress based on morphological and physiological parameters. A further purpose of this study was to establish the hydroponic method for screening rice plants under Cd stress.

MATERIALS AND METHODS

Plant materials

Sixteen cultivars of rice (cv. BRRI-62, BRRI-49, BRRI-56, BRRI-51, BRRI-72, BRRI-66, BRRI-52, BRRI-70, BRRI-54, BRRI-28, BRRI-29, Khatodosh, Sonarbangla, Parizat, Jirasail and BINA-7) were used in this study. First nine cultivars of rice seeds were collected from Bangladesh Regional Rice Research Institute, Rajshahi, Bangladesh; BRRI-28 and BRRI-29 from Mohonpur, Rajshahi; Khatodosh and Sonarbangla from Tanore, Rajshahi; Parizat, Jirasail, and BINA-7 from Saidpur.

Germination and growth conditions for hydroponic culture

Before growing, seeds were surface sterilized in 70% ethanol and 5% sodium hypochlorite for 1 and 15 min, respectively. Seeds were then rinsed five times with deionized water. Seeds were germinated on moist filter paper wetted with deionized water for 3–4 days in the dark at room temperature. Only healthy and uniform seedlings were transplanted to solution culture. A basal nutrient solution (Hoagland and Arnon 1950) was used with the following nutrient concentrations (μM): KNO_3 (16000), $Ca(NO_3)_2.4H_2O$ (6000), $NH_4H_2PO_4$ (4000), $MgSO_4.7H_2O$ (2000), KCl (50), H_3BO_3(25), Fe-EDTA (25), $MnSO_4$. 4H2O (2), $Na_2MoO_4.2H_2O$ (0.5), $CuSO_4.5H_2O$ (0.5). Plants were grown in 2 L of the aerated solution, and the environment was strictly maintained under 10 h light and 14 h dark (550–560 μmol s^{-1} per μA). The pH was adjusted to 6.3 by using NaOH or HCl. For Cd treatment 10 μM, 30 μM, 50 μM, and 100 μM $CdSO_4$ were added to the culture solution in the hydroponic system till 10 days. Non-stressed control plants were grown concurrently and harvested at the same time.

Measurement of morphological characters

Shoot height, root length, shoot fresh weight, and root fresh weight was measured on 7 days old plants grown on solution culture. Total roots developed by each plant sample were washed in distilled water to remove nutrient and then quickly blotted in tissue paper, then weighted by electronic balance. Fresh weight of shoot was directly measured by electronic balance after harvesting.

Determination of chlorophyll content in leaves

The chlorophyll concentration (*a* and *b*) of leaves was determined spectrophotometrically as described previously by Lichtentaler and Wellburn with modifications (1985). Firstly, 100 mg leaf was weighted and placed in 95% acetone in a 5 ml falcon tube. The leaf sample was then ground using mortar-pestle. The homogenate was filtered through Whatman filter and was centrifuged at 2500 rpm for 10 min. The supernatant was separated, and the absorbances were read at 666 (chlorophyll *a*) and 653 (chlorophyll *b*) on a spectrophotometer. The amount of these pigments was calculated according to the formula given by Lichtentaler and Wellburn (1985).

Statistical analysis

Statistical analyses and graphs were performed using IBM SPSS Statistics 20 and GraphPad Prism 6 software, respectively. Statistical significance was set at $P \leq 0.05$. Data represented in the Tables and Figures are means ± standard deviations (SD) of three replicates for each treatment.

RESULTS

Shoot height and root length

Shoot heights were significantly decreased in BRRI-49, BRRI-56, BRRI-28,BRRI-72, BRRI-66, Khatodosh, Parijat, BINA-7, Jirasail, BRRI-52, BRRI-70, BRRI-62, BRRI-29, and BRRI-54 supplemented with all concentrations of Cd compared with controls (Table-1). However, BRRI-51 cultivar showed no significant decrease in shoot height when plants were treated with 10μM Cd but significantly decreased in shoot height treated with 30μM Cd or greater Cd concentrations compared with the controls (Table 1). Interestingly, Sonarbangla cultivar showed no significant in shoot height up to 50μM Cd but then significantly decreased when plants were supplemented with 50μM and 100μM Cd in hydroponic conditions (Table 1).

Root lengths were significantly decreased in BRRI-49, BRRI-56, BRRI-28, BRRI-72, BRRI-66, Parijat, BINA-7, BRRI-70, and BRRI-29 supplemented with all concentrations of Cd compared with controls (Table 2). Further, BRRI-51, Khatodosh, Jirasail, BRRI-52, BRRI-62 and BRRI-54 cultivars showed a significant decrease in root length in response to 30μM Cd or greater Cd concentrations compared with the controls (Table 2). Interestingly, no significant changes in root length were observed in Sonarbangla subjected to all levels of Cd compared with control (Table 2).

Table 1. Measurement of shoot height in different rice cultivars grown without (control) and with different Cd concentrations (10μM $CdSO_4$, 30μM $CdSO_4$, 50μM $CdSO_4$ and 100μM $CdSO_4$) under hydroponic conditions. Values are means ± SD of three independent replications (n=3). Different superscripted letters (a-e) within the row indicate statistically significant differences among the treatments according to Duncan's multiple range test (P<0.05). Data were taken on 7 days old plant.

Cultivars	Treatment				
	Control	10 μM Cd+	30 μM Cd+	50 μM Cd+	100 μM Cd+
BRRI-49	8.7000±0.4359^{d}	6.0667±0.3786^{c}	5.3667±0.3786^{b}	4.8333±0.3512^{ab}	4.2667±0.2082^{a}
BRRI-56	12.9000±0.5292^{d}	8.0000±0.6245^{c}	6.5667±0.4041^{b}	6.1667±0.2517^{b}	5.2333±0.5132^{a}
BRRI-51	9.5000±0.1000^{d}	8.9333±0.5033^{d}	7.5000±0.3000^{c}	5.5333±0.5132^{b}	4.1667±1.0017^{a}
BRRI-28	8.1333±0.6429^{d}	7.0667±0.4509^{c}	5.8667±0.3215^{b}	5.4667±0.2517^{b}	4.6333±0.4163^{a}
SONARBANGLA	10.6701±0.5671^{b}	10.3456±0.4094^{b}	8.7333±0.1528^{b}	7.7667±0.6506^{a}	7.4667±0.2309^{a}
BRRI-72	11.8907±0.2087^{c}	7.1790±0.7804^{b}	5.6667±0.7638a^{b}	5.5000±0.3000a^{b}	4.7333±0.4726^{a}
BRRI-66	12.7333±0.4041^{d}	8.4000±1.0392^{c}	7.2000±0.7550b^{c}	7.3333±0.1528^{b}	5.2667±0.1155^{a}
KHATODOSH	11.8667±1.8717^{d}	8.1333±0.1528^{c}	6.7667±0.7024^{bc}	5.7667±0.6429^{ab}	4.8667±0.2082^{ab}
PARIJAT	12.6000±1.3077^{d}	8.7000±0.7000^{c}	7.5000±0.3000^{bc}	6.4000±0.1732^{ab}	5.3667±0.2887^{a}
BINA-7	11.6333±2.2591^{b}	6.6667±0.4619^{a}	5.8000±0.3000^{a}	5.6333±0.5132^{a}	4.7667±0.5859^{a}
JIRASAIL	12.4000±0.2646^{e}	8.4000±0.2646^{d}	7.3333±0.4163^{b}	6.6000±0.1732^{c}	5.3333±0.5774^{a}
BRRI-52	8.8333±0.5859^{d}	6.5667±0.4163^{c}	5.9000±0.5568^{bc}	5.2333±0.2082^{ab}	4.4333±0.5774^{a}
BRRI-70	10.9667±0.9074^{d}	7.5667±0.2082^{c}	6.7000±0.8718^{bc}	6.4333±0.0577^{b}	5.3667±0.1155^{a}
BRRI-62	10.2333±1.5373^{c}	7.5000±0.3606^{b}	5.7333±0.5508^{ab}	6.5667±0.5508^{a}	5.1000±0.1000^{a}
BRRI-29	10.0000±0.1732^{d}	6.2333±0.2517^{c}	5.9667±0.5686^{bc}	5.4333±0.5859^{ab}	4.8667±0.2517^{a}
BRRI-54	9.6000±0.5568^{d}	6.9000±0.2000^{c}	6.3667±0.1528^{c}	5.5333±0.0577^{b}	4.4333±0.4041^{a}

Shoot and root fresh weight

Shoot fresh weights significantly decreased in BRRI-49, BRRI-56, BRRI-51, BRRI-28,BRRI-72, BRRI-66, Parijat, BINA-7, Jirasail, BRRI-52, BRRI-70, BRRI-62, and BRRI-29 supplemented with all concentrations of Cd compared with control (Table 3).Also, Khatodosh and BRRI-54 showed a significant decrease in shoot fresh weight when plants were grown under 30μM Cd and 50μM Cd, respectively, in comparison with control. Again, Sonarbangla showed significant reduction in shoot fresh weight only when plants were grown under 100μM Cd compared with the plants grown without Cd supplementation (Table 3).

Table 2. Measurement of root length in different rice cultivars grown without (control) and with different Cd concentrations (10µM $CdSO_4$, 30µM $CdSO_4$, 50µM $CdSO_4$ and 100µM $CdSO_4$) under hydroponic conditions. Values are means ± SD of three independent replications (n=3). Different superscripted letters (a-d) within the row indicate statistically significant differences among the treatments according to Duncan's multiple range test (P<0.05). Data were taken on 7 days old plant.

Cultivars	Treatment				
	Control	10 µM Cd+	30 µM Cd+	50 µM Cd+	100 µM Cd+
BRRI-49	7.8667±0.7095^{c}	5.3333±0.2082^{b}	4.7000±0.2646^{ab}	4.2333±0.2517^{a}	3.9667±0.4509^{a}
BRRI-56	11.3333±0.5774^{d}	8.3333±0.3215^{c}	6.4333±0.1155^{b}	6.1667±0.2082^{b}	5.1667±0.7506^{a}
BRRI-51	8.8667±0.5508^{d}	8.3333±0.5508^{d}	7.3000±0.4359^{c}	6.1333±0.1528^{b}	5.2333±0.2082^{a}
BRRI-28	8.5000±0.7937^{b}	6.6333±1.8502^{a}	5.3667±0.4726^{a}	5.1000±0.7937^{a}	4.7667±0.4509^{a}
SONARBANGLA	11.9890±0.5800^{a}	11.5921±0.9640^{a}	10.0333±0.7371^{a}	10.9667±1.4224^{a}	10.8333±0.7638^{a}
BRRI-72	10.8700±0.5100^{c}	8.0090±0.5089^{b}	5.1000±1.3528^{a}	5.3000±0.4583^{a}	4.4667±0.3786^{a}
BRRI-66	9.3333±0.2887^{c}	7.2000±0.6083^{b}	4.7667±0.7371^{a}	4.8333±0.5774^{a}	4.5333±0.5033^{a}
KHATODOSH	9.8000±0.5196^{d}	8.6333±0.9815^{cd}	7.5667±1.2097^{bc}	6.8667±0.7767^{ab}	5.5000±0.5292^{a}
PARIJAT	12.6667±1.2583^{c}	8.6667±0.7024^{b}	7.4667±1.0017^{ab}	7.4333±0.7371^{ab}	6.4667±0.6506^{a}
BINA-7	8.5333±0.4509^{c}	5.8667±0.7024^{b}	5.3667±0.2517^{b}	5.1000±0.4583^{b}	3.9000±0.3606^{a}
JIRASAIL	9.8333±0.7234^{d}	9.2000±1.2530^{cd}	8.3000±0.2646^{bc}	7.1333±0.1155^{ab}	6.1667±0.5774^{a}
BRRI-52	8.5000±0.5000^{d}	8.2667±0.2517^{d}	7.3667±0.3215^{c}	6.4000±0.3606^{b}	5.2667±0.5859^{a}
BRRI-70	8.0667±0.0577^{d}	7.0000±0.3464^{c}	6.3667±0.2309^{bc}	5.8000±0.1732^{b}	4.4667±1.1015^{a}
BRRI-62	6.7333±0.7095^{c}	6.6667±0.0577^{bc}	6.1333±0.8145^{bc}	5.6333±0.5132^{ab}	5.0667±0.2517^{a}
BRRI-29	10.0333±1.2858^{c}	6.8333±0.4619^{b}	6.7667±0.8327^{b}	6.0333±0.5033a^{b}	4.8667±0.3215^{a}
BRRI-54	9.1667±2.6312^{b}	7.4000±1.0149a^{b}	5.3333±0.5774^{a}	5.2000±0.7550^{a}	4.9333±0.8327^{a}

Table 3. Measurement of shoot fresh weight in different rice cultivars grown without (control) and with different Cd concentrations (10µM $CdSO_4$, 30µM $CdSO_4$, 50µM $CdSO_4$ and 100µM $CdSO_4$) under hydroponic conditions. Values are means ± SD of three independent replications (n=3).Different superscripted letters (a-e) within the row indicate statistically significant differences among the treatments according to Duncan's multiple range test (P<0.05). Data were taken on 7 days old plant.

Cultivars	Treatment				
	Control	10 µM Cd+	30 µM Cd+	50 µM Cd+	100 µM Cd+
BRRI-49	0.0387±0.0049^{d}	0.0264±0.0018^{c}	0.0238±0.0015^{b}	0.0215±0.0006^{ab}	0.0203±0.0018^{a}
BRRI-56	0.0540±0.0012^{d}	0.0369±0.0023^{c}	0.0309±0.0021^{b}	0.0306±0.0015^{b}	0.0260±0.0035^{a}
BRRI-51	0.0502±0.0026^{e}	0.0415±0.0017^{d}	0.0314±0.0035^{c}	0.0269±0.0016^{b}	0.0214±0.0012^{a}
BRRI-28	0.0415±0.0040^{c}	0.0302±0.0041^{b}	0.0209±0.0013^{a}	0.0194±0.0028^{a}	0.0202±0.0020^{a}
SONARBANGLA	0.0480±0.0058^{a}	0.0471±0.0064^{a}	0.0436±0.0035^{a}	0.0380±0.0039^{a}	0.0376±0.0054^{a}
BRRI-72	0.0588±0.0064^{b}	0.0290±0.0033^{a}	0.0239±0.0030^{a}	0.0236±0.0029^{a}	0.0214±0.0024^{a}
BRRI-66	0.0553±0.0033^{c}	0.0394±0.0070^{b}	0.0377±0.0060^{b}	0.0313±0.0008^{ab}	0.0260±0.0016^{a}
KHATODOSH	0.0405±0.0043^{c}	0.0396±0.0027^{bc}	0.0358±0.0036^{abc}	0.0311±0.0082^{ab}	0.0289±0.0010^{a}
PARIJAT	0.0476±0.0034^{d}	0.0365±0.0028^{c}	0.0311±0.0007^{b}	0.0242±0.0027^{a}	0.0210±0.0003^{a}
BINA-7	0.0465±0.0106^{c}	0.0294±0.0037^{b}	0.0236±0.0030^{ab}	0.0211±0.0010^{ab}	0.0183±0.0016^{a}
JIRASAIL	0.0473±0.0008^{c}	0.0368±0.0065^{b}	0.0322±0.0019^{b}	0.0300±0.0006^{ab}	0.0245±0.0051^{a}
BRRI-52	0.0529±0.0058^{c}	0.0404±0.0025^{b}	0.0353±0.0026^{b}	0.0261±0.0033^{a}	0.0206±0.0008^{a}
BRRI-70	0.0405±0.0003^{d}	0.0278±0.0003^{c}	0.0239±0.0013^{b}	0.0224±0.0019^{ab}	0.0208±0.0009^{a}
BRRI-62	0.0388±0.0055^{c}	0.0275±0.0015^{b}	0.0246±0.0019^{ab}	0.0220±0.0010^{a}	0.0203±0.0003^{a}
BRRI-29	0.0458±0.0029^{c}	0.0292±0.0047^{b}	0.0240±0.0017^{a}	0.0201±0.0002^{a}	0.0193±0.0011^{a}
BRRI-54	0.0366±0.0064^{c}	0.0313±0.0018^{bc}	0.0291±0.0017^{b}	0.0214±0.0011^{a}	0.0205±0.0007^{a}

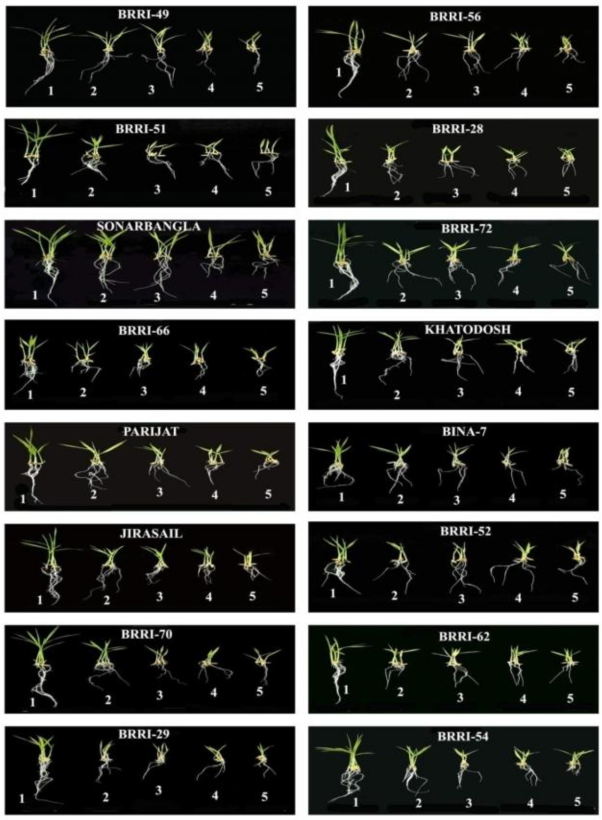

Fig. 1. The phenotype of different rice cultivars grown hydroponically with or without Cd stress for 7 days. Numeric letters 1, 2, 3, 4 and 5 indicates control, 10µM $CdSO_4$, 30µM $CdSO_4$, 50µM $CdSO_4$ and 100µM $CdS0_4$ respectively.

All cultivars showed a significant decrease in root fresh weight subjected to 10µM Cd compared with control. However, root fresh weight was significantly decreased in Sonarbangla only when plants were cultivated under 100 µM Cd compared with control (Table 4).

Chlorophyll concentrations in leaves

Concentrations of total chlorophyll (chlorophyll *a* and chlorophyll *b*) were determined in leaves of all rice genotypes grown in absence and presence of Cd hydroponic culture. No significant difference was observed in chlorophyll (*a* and *b*) concentration in BRRI-49, BRRI-28, Sonarbangla and Khatodosh under 10µM Cd compared to control (Fig. 2). However,BRRI-56, BRRI-72, BRRI-66, Parijat, BINA-7, Jirasail, BRRI-52, BRRI-70, BRRI-62, BRRI-29 and BRRI-54 showed significant decrease in chlorophyll (*a* and *b*) concentration started from 10µM Cd in comparison with non-treated control. Further, BRRI-49, BRRI-51, and Sonarbangla showed no significant reduction in chlorophyll (*a* and *b*) concentration up to 30µM Cd (Fig. 2). Lastly, all genotypes showed significant reduction in chlorophyll (*a* and *b*) concentration when plants were treated with 50 µM Cd and 100 µM Cd compared with plants grown without Cd (Fig. 2).

Table 4. Measurement of root fresh weight in different rice cultivars grown without (control) and with different Cd concentrations (10µM $CdSO_4$, 30µM $CdSO_4$, 50µM $CdSO_4$ and 100µM $CdSO_4$) under hydroponic conditions. Values are means ± SD of three independent replications (n=3). Different superscripted letters (a-d) within the row indicate statistically significant differences among the treatments according to Duncan's multiple range test (P<0.05). Data were taken on 7 days old plant.

Cultivars	Treatment				
	Control	10 µM Cd+	30 µM Cd+	50 µM Cd+	100 µM Cd+
BRRI-49	0.0115±0.0009^{c}	0.0088±0.0007^{b}	0.0098±0.0040^{b}	0.0048±0.0012^{a}	0.0037±0.0005^{a}
BRRI-56	0.0198±0.0008^{d}	0.0162±0.0008^{c}	0.0106±0.0003^{b}	0.0105±0.0004^{b}	0.0065±0.0015^{a}
BRRI-51	0.0163±0.0008^{d}	0.0124±0.0010^{c}	0.0090±0.0010^{b}	0.0059±0.0006^{a}	0.0051±0.0002^{a}
BRRI-28	0.0138±0.0024^{b}	0.0102±0.0013^{a}	0.0087±0.0017^{a}	0.0081±0.0002^{a}	0.0074±0.0014^{a}
SONARBANGLA	0.0170±0.0010^{b}	0.0117±0.0011^{b}	0.0103±0.0027^{b}	0.0097±0.0014^{ab}	0.0074±0.0021^{a}
BRRI-72	0.0164±0.0015^{d}	0.0088±0.0009^{c}	0.0064±0.0003^{bc}	0.0047±0.0005^{ab}	0.0041±0.0010^{a}
BRRI-66	0.0184±0.0023^{d}	0.0144±0.0013^{c}	0.0133±0.0009^{bc}	0.0108±0.0005^{b}	0.0079±0.0016^{a}
KHATODOSH	0.0158±0.0029^{c}	0.0095±0.0019^{b}	0.0069±0.0009^{ab}	0.0057±0.0006^{a}	0.0050±0.0007^{a}
PARIJAT	0.0160±0.0015^{d}	0.0097±0.0009^{c}	0.0096±0.0009^{c}	0.0074±0.0006^{b}	0.0053±0.0013^{a}
BINA-7	0.0164±0.0021^{c}	0.0081±0.0010^{b}	0.0070±0.0002^{ab}	0.0060±0.0002^{a}	0.0054±0.0004^{a}
JIRASAIL	0.0119±0.0007^{d}	0.0103±0.0008^{c}	0.0070±0.0004^{b}	0.0060±0.0003^{b}	0.0047±0.0005^{a}
BRRI-52	0.0145±0.0025^{c}	0.0120±0.0015^{b}	0.0108±0.0005^{b}	0.0073±0.0003^{a}	0.0067±0.0005^{a}
BRRI-70	0.0115±0.0004^{c}	0.0091±0.0002^{b}	0.0081±0.0012^{b}	0.0065±0.0006^{a}	0.0052±0.0010^{a}
BRRI-62	0.0141±0.0015^{c}	0.0105±0.0013^{b}	0.0087±0.0003^{b}	0.0059±0.0009^{a}	0.0042±0.0010^{a}
BRRI-29	0.0158±0.0015^{d}	0.0091±0.0007^{c}	0.0078±0.0006^{bc}	0.0070±0.0003^{b}	0.0054±0.0003^{a}
BRRI-54	0.0088±0.0020^{b}	0.0087±0.0018^{b}	0.0077±0.0001^{ab}	0.0060±0.0010^{a}	0.0056±0.0006^{a}

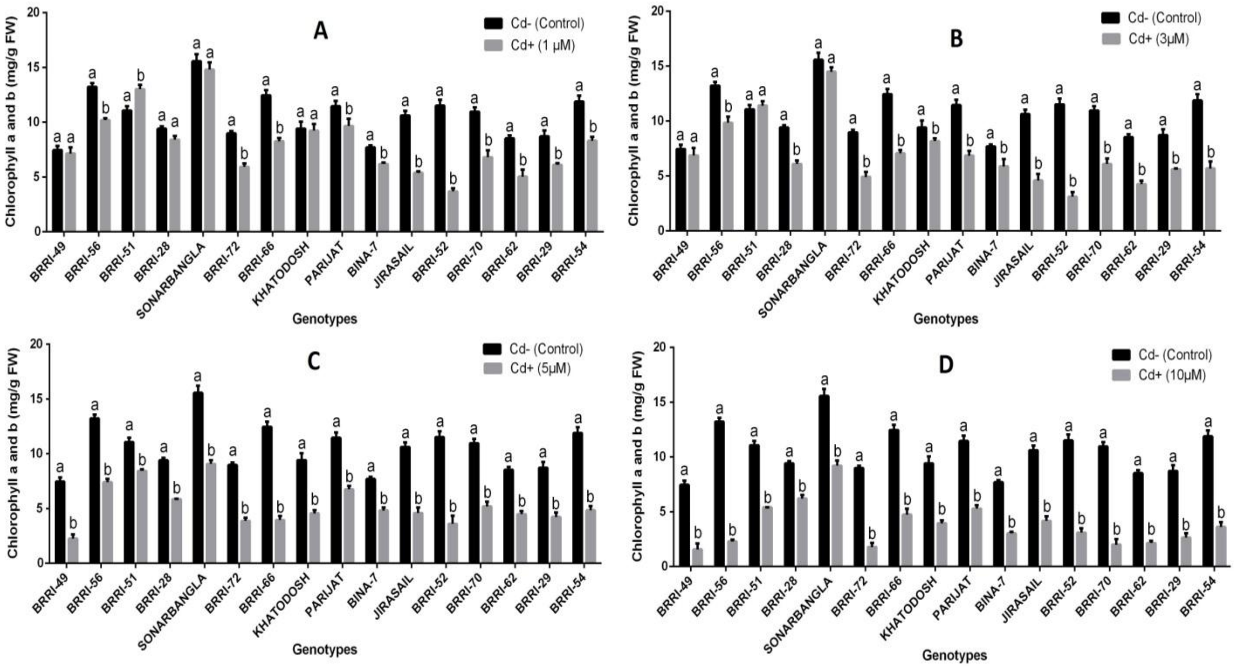

Fig. 2. Concentration of Chlorophyll a and b in young leaves in some rice genotypes grown in absence and presence of Cd hydroponic culture. (A) comparisons were done for Cd- (control) and Cd+ (10μM) conditions; (B) comparisons were done for Cd- (control) and Cd+ (30μM) conditions; (C) comparisons were done for Cd- (control) and Cd+ (50μM) conditions and (D) comparisons were done for Cd- (control) and Cd+ (100μM) conditions. Data were taken from 7 days old plant. Different letters indicate significant differences between means±SD of treatments (n=3).

Table 5. Ranking of different rice cultivars in response to Cd stress. The numerical number indicates tolerance level of rice cultivars supplemented with all concentrations of Cd compared with controls based on morphological parameters (SH=Shoot Height, RL=Root Length, SFW=Shoot Fresh Weight, RFW=Root Fresh weight and TC=Total Chlorophyll).

Cultivars	**Morphological Parameters**					**Sum[a]**	**Genotype Ranking[b]**	**Tolerance Degree[b]**
	SH	RL	SFW	RFW	T C			
BRRI-49	8.5	6.5	8.5	6	6	35.5	7	Sensitive
BRRI-56	8	8	8	8	7	39	12	Sensitive
BRRI-51	6	6	10	9	6	37	9	Sensitive
BRRI-28	8	4	7	4	7	30	3	Sensitive
SONARBANGLA	2	0	0	2	4	8	1	Tolerant
BRRI-72	5	7	4	8	6	30	3	Sensitive
BRRI-66	7	7	5.5	7.5	6.5	33.5	6	Sensitive
KHATODOSH	7.5	7.5	5.5	6.5	5	32	4	Sensitive
PARIJAT	8	6	9	7	6.5	36.5	8	Sensitive
BINA-7	4	5	7	6.5	7.5	30	3	Sensitive
JIRASAIL	10	7.5	5.5	8	7	38	11	Sensitive
BRRI-52	8	6	6	6	6.5	32.5	5	Sensitive
BRRI-70	7.5	7.5	8.5	6	8	37.5	10	Sensitive
BRRI-62	6.5	5.5	6.5	6	5.5	30	3	Sensitive
BRRI-29	8	5	7	7.5	5	32.5	5	Sensitive
BRRI-54	7	3	5.5	2.5	6	24	2	Moderate

[a]Sums were obtained from the group ranking by adding the ranking numbers at the five morphological parameters in each genotype.
[b]Genotypes were finally ranked based on the sums with the smallest sum being the most relatively tolerant.

Ranking of the genotypes

Ranking of genotypes was performed based on the score. The scoring was done on statistical significance between control and each concentration of Cd used in the hydroponic culture of rice genotypes. The greater sum indicates the sensitivity of rice genotype in response to Cd stress. According to the ranking analysis, Sonarbangla was the most tolerant genotype under Cd stress resulted in a minimum score (8), followed by BRRI-54 (24). The rest of the genotypes showed 30 or more scores in the analysis and thus, we categorized them as sensitive to Cd stress (Table 5).

DISCUSSION

The growth and biomass in rice genotypes showed wide changes across five different level of Cd. Visual symptoms due to Cd stress were compared with the actual morpho-physiological traits under Cd and normal hydroponic conditions. It was observed that shoot height and root length was predominantly affected due to Cd stress in rice genotypes. However, Sonarbangla showed unchangeable shoot height in comparison with control up to 50µM Cd and was graded as tolerant which scored 2 (Table 5). Depending on the tolerance level in response to different Cd concentration, rest of the rice genotypes scored from 4 to 10. Measurement of shoot height as the morphological marker was reported to be useful for screening salt tolerant rice landraces (Ali et al. 2014).

It is evident from the results of a present study that the root length showed remarkable variations in all of the genotypes subjected to different Cd concentrations used in hydroponic culture. Thus, tolerance to various dose of Cd was scored differently among the genotypes. Most of the genotypes were affected due to 10µM and therefore, scored 4 to 7.5 (Table 5). Interestingly, Sonarbangla scored 0 for root length as this genotype was not damaged in any of the Cd stress.

Being consistent with plant length, root and shoot fresh weight was not remarkably changed up to 50µMCd stress in Sonarbangla, confirming that Cd tolerance does exist in this genotype. The severity of damage was also reported in other genotypes while observing root and shoot fresh weight. Roots are considered to be critical in heavy metal tolerance as roots give the first barrier in metal uptake, excretion, and retention (Ramos et al. 2002).

Chlorophyll concentrations (*a* and *b*) showed distinct variations among the genotypes under different Cd treatments. BRRI-49, BRRI-51 and Sonarbangla were able to maintain chlorophyll level up to 30µM Cd. However, the deleterious effects of Cd may result in a significant decrease in photosynthesis rate seedlings that leads to a shortage of assimilating onto the developing organs in other rice genotypes. Chlorophyll is a parameter, has been used for screening several species including corn (Dwyer et al. 1991), wheat (Reeves et al. 1993) and field peas (Yakop 2008).

Score based on morphological features is related well to the physiological status of the plants and proved the reliability of Cd stress as a selection for rapid screening of large breeding material. Cd tolerance is not a function of a single organ or plant attribute but it is the product of all the plant attributes. Therefore a genotype exhibiting relative Cd tolerance for all the plant attributes may be an ideal one. This is supported by the overall tolerance of Sonarbangla in different root and shoots features observed in this present study. Findings of this study also bring the message to the farmers for genotypic selection where Cd contamination is a problem. Further, this genotype may be used as donors for further improvement of rice through a breeding program.

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