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MORPHOLOGICAL AND BIOCHEMICAL RESPONSES OF DIFFERENT RICE VARIETIES EXPOSED TO CADMIUM STRESS

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A hydroponic experiment was conducted to observe the effects of various cadmium (Cd) levels (0, 50, 100 µM) on morphology and biochemical responses of nine different rice varieties. Initially, seeds were sown in sand in plastic pots. After 16 days of sowing, uniform seedlings were transferred to hydroponic set-up containing Hoagland solution and continuous aeration was provided with aeration pump. After six days, plants were applied the Cd stress for four weeks, then plants were harvested. Plants growth, biomass, photosynthesis, oxidative stress markers contents, antioxidant enzymes activities and Cd uptake in both root and shoot were measured. Results revealed that Cd negatively affected the plant growth as well as photosynthesis; while, oxidative stress and Cd uptake was increased with increasing Cd stress in all nine rice varieties. From this study, it was found that among all nine rice varieties, the Chenab variety was found most tolerant; while, the Kisan Basmati was found most sensitive variety against Cd stress. However, future studies are needed in order to investigate the detailed mechanism of Cd in different varieties of rice.

Keywords: Rice, cadmium, plant growth, toxicity, oxidative stress, antioxidant machinery.

INTRODUCTION

Heavy metals are not only toxic to plants as well as humans but also their nature is tenacity. Their toxicity depends on the enhanced level of environmental pollution (Nagajyoti *et al.*, 2010; Aravind *et al*., 2020; Chiao *et al*., 2019). The major problem in now a days is the enhanced arable soils pollution with cadmium (Cd). This affect the human health as well as the quality of food (Rizwan *et al*., 2017a; Chiao *et al*., 2019). Because of greater accumulation as well as production in soils, Cd attained much attention as compared to any other heavy metals (Rehman *et al*., 2015). So, Cd is considered as one of the greatest environmental pollutants (Yang *et al*., 2017; Chen *et al*., 2018). It exists all over the world, but mainly in Asia (Wang *et al*., 2016; Naseem *et al*., 2020). It mainly found in the rice (*Oryza sativa* L.). Through plants, it can be easily taken up because Cd has tended to sustain in the solution of soil, non-degradable as well as highly mobile (Ahmad *et al*., 2020). It can be easily taken up by food crops (Qayyum *et al*., 2017; Zhang *et al*., 2020). Through interfering the ion uptakes, for example, magnesium, iron as well as calcium, cause the imbalances in nutrient (Babu and Nagabovan, 2017; Bashir *et al*., 2018). By the production of ROS, the results in exposure of Cd in oxidative stress in plants (Farooq *et al*., 2016; Rizwan *et al*., 2019). In plants, to reduce the concentration of Cd especially in food crops such as rice, it has indispensable to carry out any strategy (Rizwan *et al*., 2016a; Cui *et al*., 2017). Therefore, a number of strategy have been utilized, for example, liming (Qayyum *et al*., 2017; Chen *et al*., 2018), inorganic and organic techniques (Rehman *et al*., 2015; Cui *et al*., 2018), phytoremediation (Li *et al*., 2009), utilization of rice with less Cd accumulation (Rizwan *et al*., 2016a) as well as management of soil moisture (Li and Xu, 2015). But all these techniques take too much time as well as the outcomes was not feasible (Cui *et al*., 2017; Rizwan *et al*., 2017b; Khasanah and Rachmawati, 2020).

At world level, the major cereal crops must be considered the rice. More than 50%, it is consumed as staple food (Hawksworth, 1985; Anjum *et al*., 2019). Once a day, the main source of Cd intake is considered the rice that human beings mainly utilize grains (Rizwan *et al*., 2016b). Generally, the utilization of rice exceeds other than cereal crops, for example, wheat, that enhanced the Cd bioaccumulation factor (Arao *et al*., 2009).

In the absence of oxygen as well as in flooded paddy fields, the rice is cultivated as staple crop. In this way, arsenate is mobilized in contrast to aerobic environments (Williams *et al.*, 2005; Abbas *et al.*, 2018). The arsenite (AsIII) availability/mobility ratio is higher than the arsenate (AsV) in flooded paddy soils. This may be due to the reductive dissolution process of iron minerals such as hydroxides as well as oxides (Niazi *et al.*, 2011; Shakoor *et al.*, 2018). This reductive dissolution process exists under the absence of oxygen that increases the bioavailability of arsenate to rice plants. Moreover, those who depend on rice as their daily meal, leads to greater risks of their life because the "As-contaminated ground water" for the production of rice leads to greater diseases (Williams *et al.*, 2006). Herein, this study exposure the effect of Cd of different varieties of plants such as Chenab, KSK 434, Basmati 515, Punjab Basmati, Punjab, Noor Basmati, Super Basmati, Sarshar as well as Kisan Basmati. This study correlates the Cd effect and their accumulation over rice varieties, and we check out responsive study against all these varieties.

MATERIALS AND METHODS

Seed collection and plant culture**:** Seeds of nine different rice varieties were obtained from Ayub Agriculture Research Institute, Faisalabad, Pakistan. Nine rice varieties used for present experiment were V1 = Chenab, V2 = KSK 434, V3 = Basmati 515, V4 = Punjab Basmati, V5 = Punjab, V6 = Noor Basmati, V7 = Super Basmati, V8 = Sarshar and V9 = Kisan Basmati. Seeds of these varieties were surface sterilized with solution of dilute acid (H_2O_2, 10%), followed by washed with distilled water. Plastic pots were filled with sterilized sand and seeds were sown in this moist sand at room temperature.

Hydroponic experiment**:** Hydroponic experiment was conducted in Toxicology and Environmental Chemistry Laboratory, Department of Environmental Sciences and Engineering, Government College University Faisalabad, Pakistan. After 16 days of sowing, uniform seedlings were transplanted to hydroponic setup at thermo-pole sheets after washing the plants roots with tap water followed by distilled water to remove the sand. One hydroponic container had 50 L water with half-strength Hoagland solution. The Hoagland's solution containing 2.5 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 0.5 mM KCl, 0.5 mM KH_2PO_4, 0.1 µM $FeCl_3$, 0.2 µM $CuSO_4$, 1 µM $ZnSO_4$, 20 µM H_3BO_3, 0.005 µM H_2MoO_4 and 2 µM $MnSO_4$ (Sallah *et al.*, 2017). After 1 week, containers were refilled and full strength Hoagland solution was applied and was changed every week. After six days of transplantation, Cd treatments (0, 50, 100 µM) were applied by following completely randomized design (CRD). Continuous aeration was provided with aeration pump. The pH was checked and maintained (6.8) every 2^{nd} day with NaOH and H_2SO_4.

Rice harvesting**:** Plants were harvested after four weeks of the application of Cd treatments. Plants were washed with tap water followed by distilled water and were separated into ifferent parts. Root, shoot lengths and leaf area was measured. After oven drying, root and shoot dry weights were measured.

Chlorophyll, carotenoids and gas exchange parameters: For estimation of chlorophyll and carotenoids contents, fresh plant samples were collected. For this purpose, an extract was obtained by digesting 0.5 g plant sample into 80% acetone solution. Concentrations were obtained at various wavelengths on spectrophotometer by following the protocol of Lichtenthaler (1987). The measurement of gas exchange parameters such as water use efficiency, stomata conductance, transpiration rate and photosynthetic rate was estimated by IRGA (LCA-4, Analytical Development Company, Hoddesdon, England).

Oxidative stress parameters and antioxidant enzyme activities: For estimation of oxidative damage, the contents of oxidative stress markers such as electrolyte leakage (EL), malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were measured according to the methods as described previously (Jana and Choudhuri, 1981; Heath and Packer, 1968; Dionisio-Sese and Tobita, 1998). Plant samples were crushed in given molarity of phosphate buffer and liquid N_2. A supernatant was obtained after centrifuging the samples for 15 minutes at 4°C. Afterwards, readings were obtained at various wavelengths on spectrophotometer.

In order to estimate the antioxidant enzymes activities, the concentrations of antioxidants enzymes such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT) were measured. After obtaining the supernatant as described above, the measurement was taken at 560 nm and 470 nm on spectrophotometer (Stalwart, Germany) for SOD and POD respectively, following the protocol of Zhang (1992). For APX and CAT, Nakano and Asda (1981) and Aebi (1984) protocols were followed respectively.

Measurement of Cd concentration**:** For estimation of Cd concentration in root and shoot of rice plants, the dried samples were grinded and digested on hot plate in combined solution of $HClO_4$ and HNO_3 with ratio 1:4 (Rehman *et al.*, 2015, Kashif *et al.*, 2020, Hussain *et al.*, 2020). Afterwards, samples were filtered and the final volumes were obtained for each sample. The measurement of Cd concentration was made by running the samples at atomic absorption spectrophotometer.

Statistical analysis: All values reported in this study was mean of at least three replicates. The data was analyzed using a statistical package, SPSS version 16.0 (SPSS, Chicago,IL). A one-way variance analysis (ANOVA) was carried out, followed by the Duncan's multiple range test to determine the significant difference between means of treatment.

RESULTS

Plants growth and photosynthesis**:** The current study was conducted to observe the effects of cadmium (Cd) on morphophysiology and plants photosynthesis of nine rice varieties grown hydroponically. Cadmium effect was observed on shoot and root length, shoot and root biomass, leaf area and number of leaves (Fig. 1). Cadmium reduced plant growth and biomass in all varieties, however, this reduction was variety dependent. With increasing Cd concentration, plants growth and biomass decreased with dose additive manner. Likewise, the plant growth and biomass, the plants chlorophyll and carotenoids contents as well as photosynthesis also affected by Cd stress in all rice varieties (Fig. 2 and Fig. 3). Among different rice varieties, the Chenab variety was found most tolerant; while, Kisan Basmati was found most sensitive variety against Cd stress.

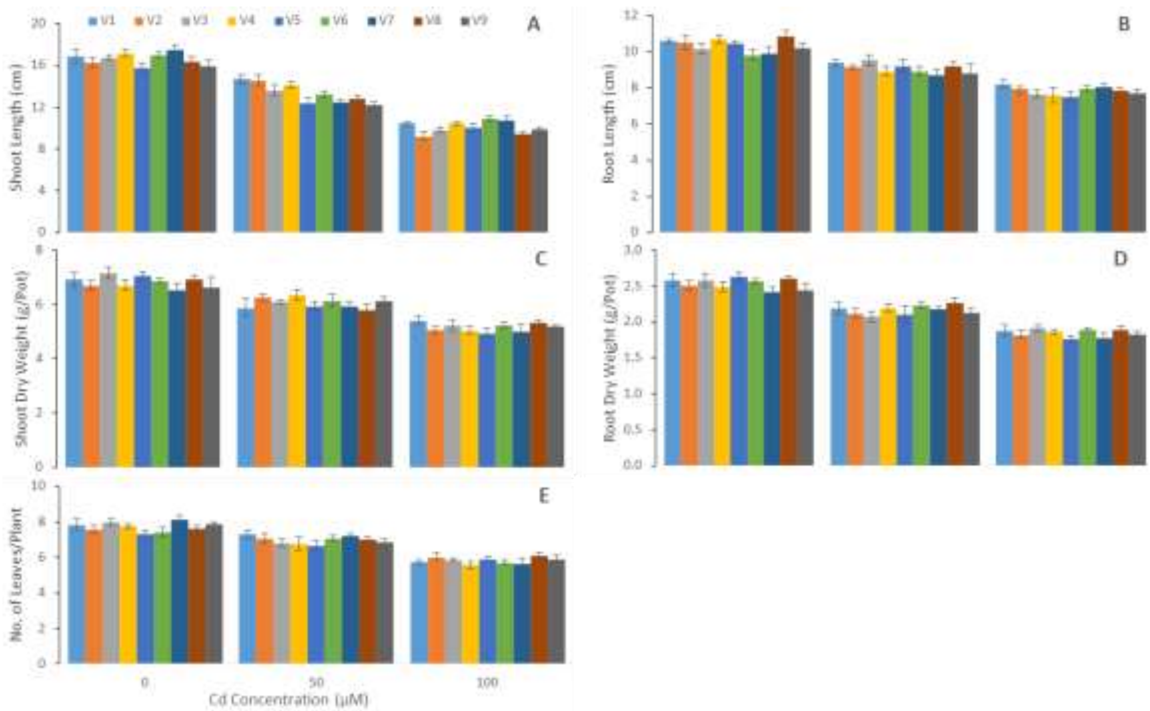

Figure 1. Effect of different Cd concentrations (0, 50, 100 µM) on shoot and root length, shoot and root dry weight and number of leaves per plant of different rice varieties grown hydroponically. Values are the means of three replicates along-with standard deviation. Different lower case letters indicate significant differences among different treatments at *P ≤ 0.05*.

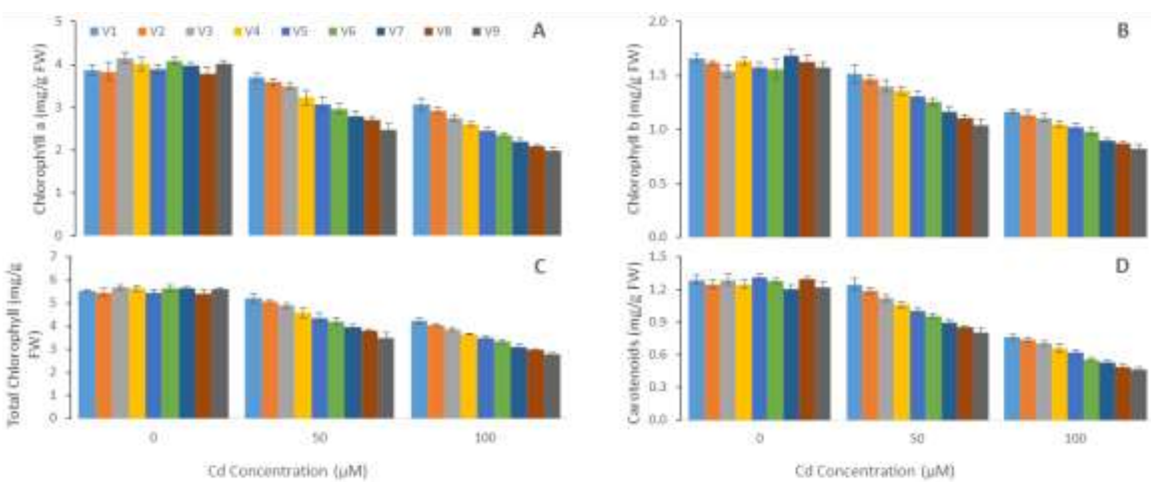

Figure 2. Effect of different Cd concentrations (0, 50, 100 µM) on chlorophyll a, b, total and carotenoid contents of different rice varieties grown hydroponically. Values are the means of three replicates along-with standard deviation. Different lower case letters indicate significant differences among different treatments at *P ≤ 0.05*.

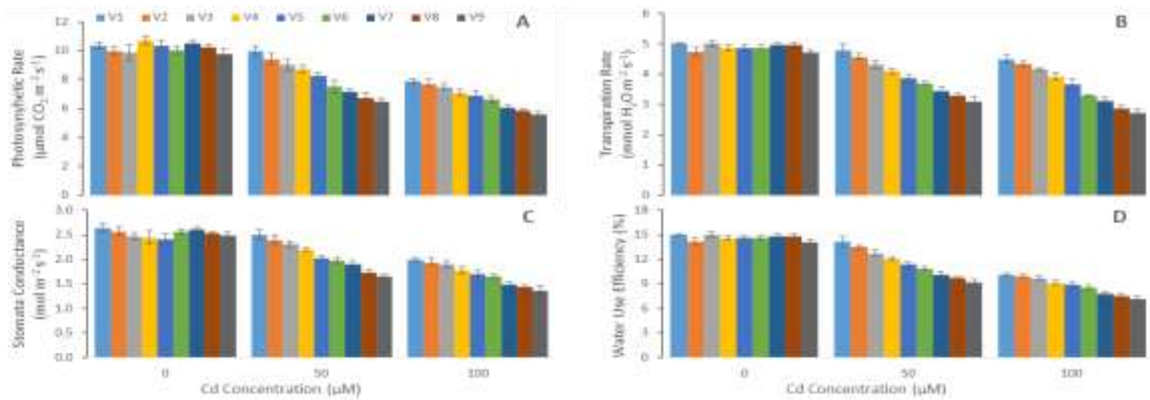

Figure 3. Effect of different Cd concentrations (0, 50, 100 µM) on photosynthetic rate, transpiration rate, stomatal conductance and water use efficiency of different rice varieties grown hydroponically. Values are the means of three replicates along-with standard deviation. Different lower case letters indicate significant differences among different treatments at *$P \leq 0.05$*.

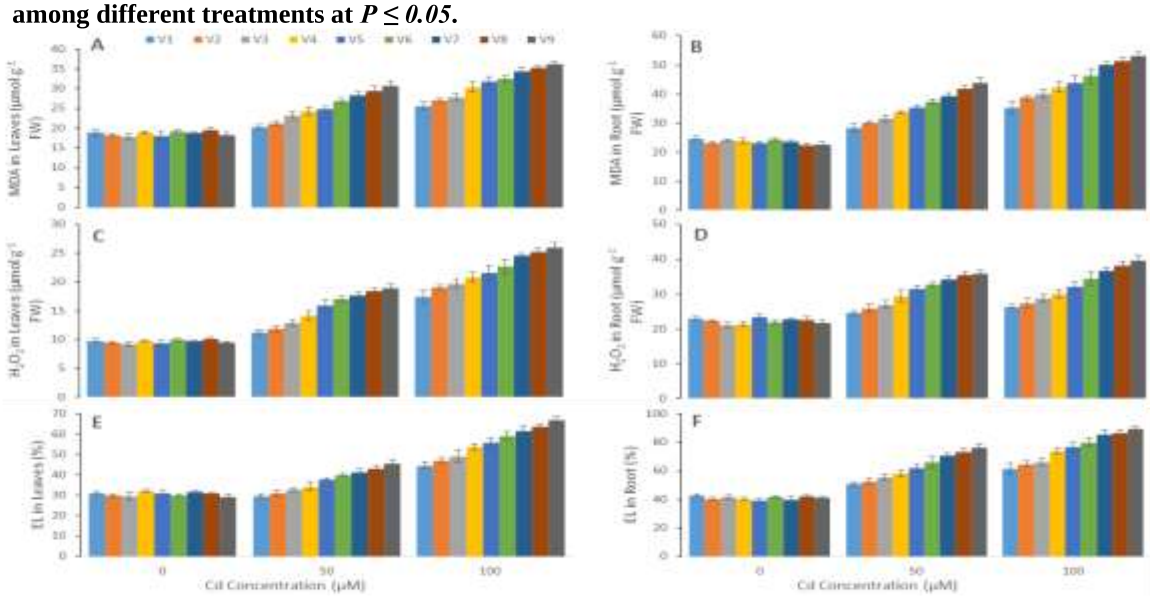

Figure 4. Effect of different Cd concentrations (0, 50, 100 µM) on EL, H_2O_2 and MDA both in root and shoot of different rice varieties grown hydroponically. Values are the means of three replicates along-with standard deviation. Different lower case letters indicate significant differences among different treatments at *$P \leq 0.05$*.

***Oxidative stress markers*:** Electrolyte leakage, MDA and H_2O_2 are plant stress markers, indicating the level of stress induced in plant under stressful environment. So, the contents for above mentioned stress markers were evaluated in root and shoot of all nine rice varieties. Cadmium stress affected the contents of mentioned stress markers in all rice varieties in different ways according to their sensitivity (Fig. 4). The Cd toxicity increased with increasing Cd concentration, resulted in enhanced oxidative stress and the contents of stress markers both in shoot and root of rice plants. Under Cd stress, the Chenab variety showed minimum values for stress markers; however, Kisan Basmati showed maximum values or all stress markers, as compared with the respective control plants. ***Antioxidant enzymes activities*:** Activities of the antioxidant enzymes (SOD, POD, CAT and APX) are the indicators of the plant defense system induced against any type of biotic and abiotic stress. These enzymes activities both in root and shoot significantly affected under Cd stress (Fig. 5). In our study, enzymes activities initially decreased at lower level of applied Cd stress (50 µM) but significantly increased at higher concentration of applied Cd stress (100 µM). While, talking about the enzymes activities in different varieties, the Chenab variety contained lowest and Kisan Basmati contained highest contents.

***Cadmium concentration*:** In order to see the uptake of Cd in different rice varieties under various levels of Cd, the Cd concentration in plant root and shoot was estimated. The results showed that Cd uptake both in plant root and shoot was linearly increased with increasing level of Cd (Fig. 6). Under Cd stress, the Chenab variety contained lowest Cd concentration as compared to other varieties, indicating that

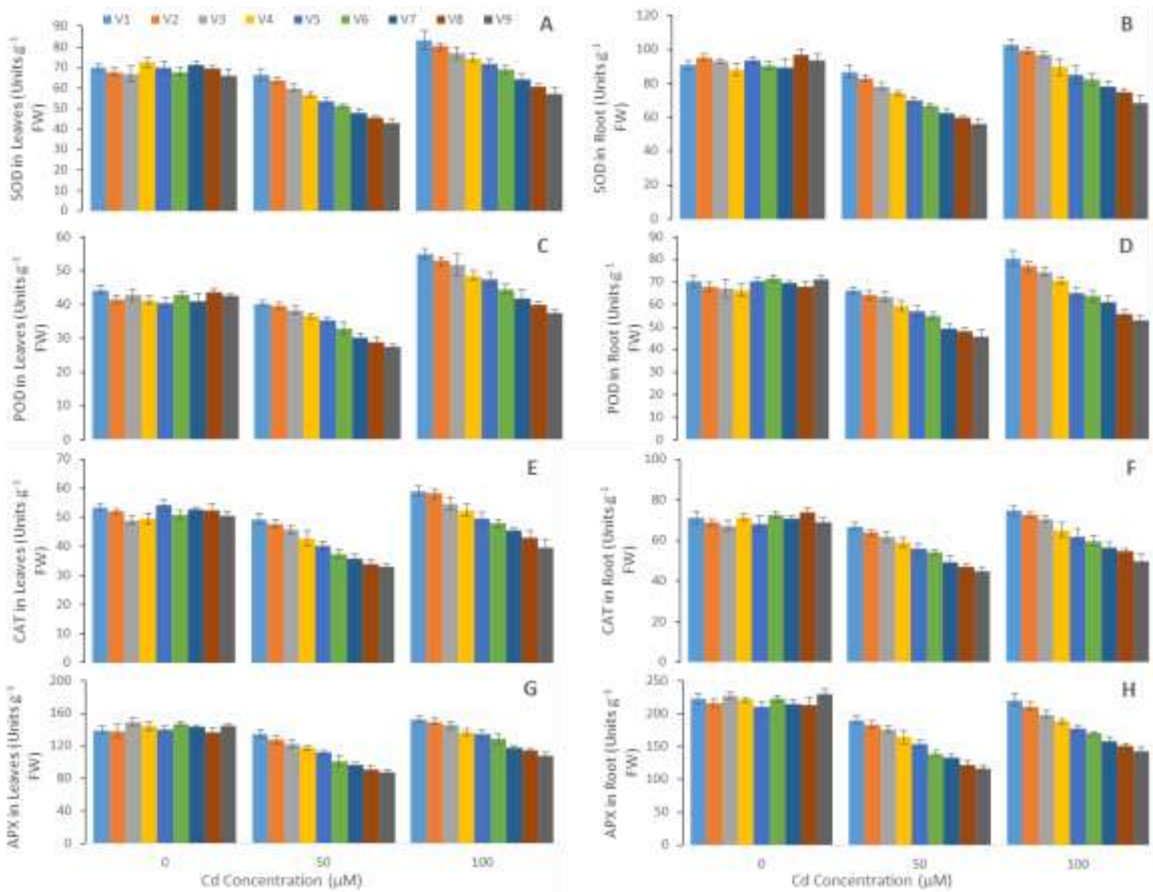

Figure 5. Effect of different Cd concentrations (0, 50, 100 µM) on SOD, POD, CAT and APX both in root and shoot of different rice varieties grown hydroponically. Values are the means of three replicates along-with standard deviation. Different lower case letters indicate significant differences among different treatments at *P ≤ 0.05*.

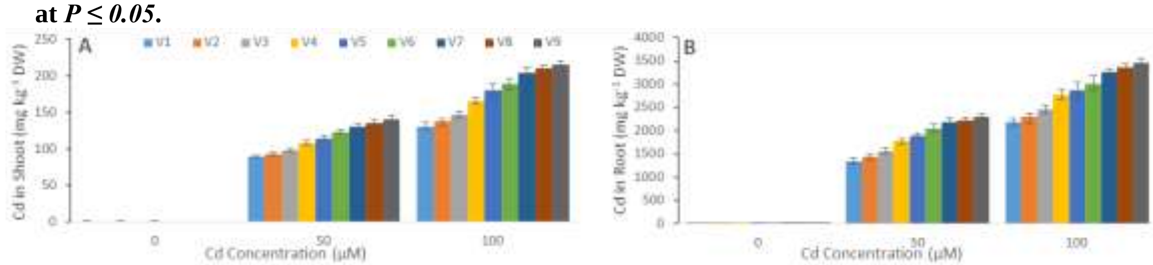

Figure 6. Effect of different Cd concentrations (0, 50, 100 µM) on Cd uptake both in root and shoot of different rice varieties grown hydroponically. Values are the means of three replicates along-with standard deviation. Different lower case letters indicate significant differences among different treatments at *P ≤ 0.05*.

Chenab is the most tolerant rice variety against Cd stress. On the other hand, Kisan Basmati showed highest Cd concentration than that of other used varieties, showing that Kisan Basmati is the most sensitive variety against the Cd stress.

DISCUSSION

It was observed that cadmium (Cd) availability in the growth medium was reflected by the content of this metal in plant. Very harmful effects were faced to plants when the level of Cd was increased in the soil (Luo *et al.,* 2019). The techniques evaluating the tolerance of heavy metals had basis on the measurement of the elongation of root of plant that faced exposure to the greater levels of the toxic ions (Jiang *et al.,* 2019). The indication of the tolerance of plants towards the heavy metals was carried out by the use of rate of root elongation, number and length of roots and their biomass. Our prior research had analysis that their difference in genotype to respond towards the toxicity of cadmium can be efficiently indicated by the value of SPAD (Yang *et al.,* 2019), and the suggested value of SPAD can help in identifying and characterizing the novel new species that are tolerant to metals. This study focuses in the determination of the value of SPAD, height of plant, biomass, volume and length of root as well as the analysis of major variation between the varieties in these parameters. Relatively, the value of SPAD was decreased much with the value of weight being 0.5, and the arrangement integrated on the basis of formula was used for measuring the tolerance of heavy metal: "integrated score 1⁄4 SPAD value 0:5 þ plant height 0:1 þ root length 0:1 þ root volume 0:1 þ dry weight 0:1 þ tillers 0:1" (* absolute reduction values in the parameter of growth in relevance to controls). So, the varieties of barley would score negative for the tolerance of Cd. Resultantly, out of total 105 varieties taken in the experiment of initial selection, ten and five varieties were finally selected as the varieties of tolerant and sensitive nature, with bottom ten having lowest and on the other hand, top five with highest integrated score respectively. Further, the secondary experiment of selection observed the simultaneous trends of change in response to the toxicity of Cd based on variety variation. The varieties Weisuobuzhi, Jipi 1 and Dong 17, Suyinmai 2, respectively were finalized as being the varieties of Cd-tolerant and Cd-sensitive nature. There was found substantial variety variation in the concentration of Cd, in which Weisuobuzhi possessed highest concentration of cadmium in tissues of plants while Jipi 1 had the lowest, although both of them were found to exhibit high tolerance of Cd regarding the parameter of the growth of seedlings. Recently, Song *et al.* (2019) carried out the analysis that metal tolerance is actually resulted from complexes of metals with inorganic compounds, organic acids and phytochelatins, specifically when hyper-accumulators are present, that help in preventing the Cd to interfere with the areas of cellular metabolism that are sensitive in nature. The Jipi 1 variety having greater content of Cd in the roots and lower content in shoot, was found to show prevention in the Cd translocation from root to shoot. Moreover, there was increase in the contents of copper and iron found in Jipi 1 shoots, comparative to other three varieties that indicated the possible role of iron and copper in the prevention of the translocation of Cd. According to previously carried out studies, there is interaction between Cd and zinc in plants for the uptake as well as distribution (Almeida *et al.,* 2019). This was suggested that there might be the effect of zinc in Cd uptake and its translocation in young plants from root to shoot. In this work, the two varieties (Dong 17 and Suyinmai 2) that are sensitive in nature, were found to possess increased decline in the content of zinc in root and shoot comparative with two varieties that are tolerant in nature. Cui *et al.* (2019) observed the decreased in Cd bioavailability in soil by zinc. Rizwan *et al.* (2017) found interference of zinc in Cd distribution in spinach and lettuce. Nevertheless, there is still no complete understanding of the reason of response as well as the related mechanism. In this study, the selection of ten genotypes of pea was done that possessed different sensitivity levels of Cd, and this selection was done based on previous results that were concerned with genetic alteration between 99 genotypes of pea. The characterization of the tolerance to Cd in the genotypes of pea plant during the growth in the culture of sand was done in first study and it comprised of the possession of heavy metals from soil that was contaminated (Ahemed *et al.,* 2019). A correlation was found in the results of studies on the growth of pea in the culture of sand and hydroponics in the experiments of short-term nature with the observations of previous studies and it further gave elaboration about the difference of the response of growth with the selected genotypes towards the toxic Cd. The production of biomass was inhibited by Cd in greater influence in the roots as compared to shoots, and in the culture of sand, there was found a contrary effect on the production of biomass. Ostensibly, the design of experiment and the conditions of growth change the toxicity of Cd, and this is dependent on the availability of Cd, the age of plant and its treatment duration. However, the correlation between the shoot and root TIs if the studied genotypes was positive, "the two genotypes, 3273 and 7128", were found to offer a striking property in the response of growth. A much greater shoot TI as compared to that of root was shown by the genotype 3273. On the other hand, a lower shoot TI was shown by genotype 7128; having greater root TI comparative to other genotypes of sensitive nature. It might be due to following reasons (i) these genotypes may have the prevailing of various Cd tolerance mechanisms and (ii) in the study of toxicology, there might be some deficiency in determining the shoot or root TI for the evaluation of plant tolerance of Cd. There is agreement between these results and that of other researchers, which describes the presence of intraspecific changes in the genes of various legume species to tolerate the heavy metals like manganese, zinc and (Jebara *et al.*, 2019; Hristozkova *et al.*, 2019; Zhan *et al.,* 2020, Brookshier *et al.,* 2018).

Conclusion: Our results revealed that Cd significantly affected the rice growth, biomass, photosynthesis under hydroponic condition. Cadmium induced the oxidative stress in plants and in response to this the activities of antioxidant enzymes were also enhanced. Cadmium uptake and translocation was enhanced with increasing Cd dose in a dose additive manner. In this study, it was found that among all nine rice varieties, the Chenab variety was found most tolerant; while, the Kisan Basmati was found most sensitive variety under Cd stress. However, future studies are needed order to investigate the detailed mechanism of Cd in rice.

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