Effects of Cadmium Stress on Antioxidant Enzymes and Osmolyte Accumulation in Aromatic Rice Seedlings

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Abstract: *Cadmium(Cd) has been proven to affect plant growth and development. Experiment was therefore, conducted to examine the influence of Cd on aromatic rice cultivars at tillering stage. The experiment was arranged in a randomized complete block design comprising three different aromatic rice cultivars (Meixiangzhan 2, Xiangyaxiangzhan and Guixiangzhan) and three Cd levels (0, 50 and 100 mg/kg^{-1} soil). Antioxidant enzymes (Superoxide dismutase (SOD), Peroxidase(POD), and Catalase(CAT)), osmolyte accumulation (proline, protein, total sugar, amino acid), lipid peroxidation (in terms of melanodialdehyde(MDA)) and growth parameters (plant height, and total dry matter content) and Cd uptake in rice cultivars exposed to different Cd levels were examined. The results showed that, with increased levels of soil Cd toxicity, activities of SOD and MDA contents increased, while activities of POD and CAT enzymes, as well as osmolyte accumulation and dry matter contents decreased for all cultivars. Cd uptake in roots and in shoots of all the cultivars increased with increased soil Cd contents. Uptake was higher in roots than in shoots with Meixiangzhan 2 showing the highest uptake in both roots and shoots followed by Xiangyaxiangzhan and Guixiangzhan cultivars. The results suggested that soil Cd toxicity have negative consequences on rice growth. Rice roots accumulated higher Cd than shoots and uptake varies amongst cultivars. Conclusively, Cd toxicity impaired early growth in rice by affecting physio-biochemical attributes, however, Guixiangzhan variety performed better than the other two varieties.*
Key Words; *Cadmium effects, Rice, Uptake, Antioxidants Enzymes, Osmulyte accumulation*

I. Introduction

Rice production is being altered by a number of abiotic stresses including heavy metals. Cadmium is among the most toxic heavy metals deposited in agricultural soilsthrough natural and anthropogenic means like; the application of sewage sludge containing Cd contents, phosphate fertilizers application and other waste disposal as well as metal smelting (Rizwan et al. 2012; Douay et al. 2009). Cd toxicity in agricultural soils is a serious threat to crop production worldwide (Rizwan et al., 2016). Even in low concentration, Cd becomes highly toxic to both growing plant as well as animalsdue to its non-essential form in living organisms, and thus affects rice seedlings morphologically, physiologically and biochemically during growth. Song et al. (2015) reported that Cd can be taken up by rice roots and then translocated to shoots and grains of growing rice. The most common visible symptoms of Cd toxicity in growing rice plants are; retardation of plant growth, chlorosis andeventually plant death.

High Cd accumulation in rice consumed by animals especially humans, has several health implications such as cardiac failure, anaemia, cancer, hypertension, emphysema, proteinuria, cerebrovascular infarction, damage to the lungs, renal dysfunction in eyes, and osteoporosis (Ashraf et al., 2015; Sebastian and Prasad., 2015). Cd translocation from soil to shoot and finally to the edible parts of rice(grains) is the easiest pathway by which increasing Cd exposure opportunity to human beings is catalysed (Römkens et al . 2009). Research has shown that, nearly sixteen percent (16%) of the agricultural soils in China have been polluted by varying heavy metals, of these, approximately 1.3×105 hm^2 of these soils have been polluted by varying degrees of Cd, and agricultural products polluted by Cd resulting to 1.46×108 kg, including 50 000 tons of rice (Xu et al. 2014).

Rice (Oryza*sativa L.*), is a major cereal crop cultivated and consumed worldwide, it's the second most important cereal crop after wheat in terms of area cultivated and consumption rate. Due to the level of importance attached to rice, it therefore, becomes eminent to understand how different aromatic rice cultivars

perform under varying levels of cadmium toxicity. Rice growth and development is most eminent during the vegetative growth stage where seedlings development is initiated. Rice growth at tillering stage comprises roots development for nutrients uptake,hypocotyl elongationand enzymes activation for mobilizing stored energy and nutrients as well as photosynthetic processes. Cd stress has been proven to have some morpho-physiological and antioxidants effects in rice seedlings at the early growth stage (vegetative stage) especially in paddy polluted soils. Reports have shown reduction in rice growth and biomass, which might possibly be as a result of different Cd-mediated toxicity mechanisms in rice (Srivastava et al. 2014). Other studies have reported that the toxic effects of Cd increased rice seedlings oxidative stress by releasing reactive oxygen species (ROS) like malondialdehyde (MDA) contents, hydrogen peroxide (H_2O_2) and electrolyte leakage which affects rice growth and performance(Yu et al. 2013; Srivastava et al. 2014). Cadmium toxicity also altered leaf and root ultrastructure and caused structural damages to photosynthetic apparatus of rice.

From several other studies conducted, marked differences in Cd uptake and translocation among plant species as well as among cultivars within the same species were observed (Liu et al. 2007; Grant et al. 2008; Sebastian and Prasad, 2015).

Most research conducted mainly focused on rice performance at maturity when subject to Cd stress conditions, little is known regarding different aromatic rice genotypes performances at tillerering stage when grown under cadmium toxic soils. This study looked at variation of Cd uptake in scented rice roots and translocation to straws as wellas activities ofantioxidantenzymes, osmolyte accumulation and growth parameters during the vegetative stage in rice subjected to Cd stressed conditions. The results will serve useful purposes for understanding the performance of different aromatic rice cultivars under varying cadmium stress.

II. Research Design

Soil Preparation and Pot Experiment

Pot experiment was conducted in rain protected greenhouse under open-air conditions at the experimental research farm (College of Agriculture, South China Agricultural University (SCAU)), Guangzhou city (23°14′ N, 113°37′ E, 20 M altitude) during 2015/2016 cropping years. The pots were arranged in a two Factor Factorial Experiment with Completely Randomized Design, each treatment replicated three times. Paddy soil from the research farm was collected to a depth of 20 cm and then air dried, after air-drying, the paddy soil was grinned and sieved through a 4 mm sieve, mixed thoroughly and filled in the pots (25 cm diameter by 30 cm height). About 10 kilograms of soil was filled in each pot and Cd in the form of $CdCl_2.2$ $1/2$ H_2O was added to the soil to obtain the following Cd levels as: a control with no added Cd (Cd 0), 50 mg Cd/kg of soil added (Cd 1), and 100 mg Cd/kg of soil added (Cd 2). The pots once filled with soil were thoroughly mixed with the exact amount of Cd ($CdCl_2.2$ $1/2$ H_2O) based on the treatment and kept for 15 days before the rice seedlings were transplanted in them. Soil used for the experiment was analyzed before use and was found to contain 4.96 mg/kg Cd content, 5.92 pH level, 18.73 g/kg organic matter contents, while total NPK was 0.81, 0.9 and 16.79 g/kg, and available NPK 69.15, 10.15 and 109.62 mg/kg respectively.

Rice Cultivars Preparation

Three aromatic rice varieties, Meixiangzhan 2, Xiangyaxiangzhan, and Guixiangzhan, were secured from the Department of Crop Science and Technology, College of Agriculture, South China Agricultural University (SCAU)), Guangzhou. Rice seeds were first soaked in deionized water for about 48hrs. at room temperature (20-25 ℃) and later nursed on March 3rd, 2015 in uncontaminated soil using parachute trays under moist conditions. After 15 days (29th March, 2015), the seedlings were transplanted into the pots (4 seedlings per pot). The pots were maintained under flooded conditions of about 2-3 cm water level above the soil surface during the whole vegetative growth period. Required rates of fertilizers were split applied as basal fertilizer and during the growth period after transplanted seedlings had maintained growth stability. Entire rice was harvested and samples collected after six weeks. The samples were separated in to fresh samples stored in refrigerators at required temperatures for enzymes analysis, while the other set was oven dried at 80°C for analysis of plants biomass accumulation and Cd uptake in straw and root.Antioxidant enzymes (superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT)) and Malondialdehyde (MDA) Detection.

Enzymes were extracted following Cho and Seo (2005) method. In which rice straw were homogenised using a mortar and pestle with a 0.05 M sodium phosphate buffer of pH 7.5 containing Mm ethylenediaminetetraacetic acid (EDTA) and 1% polivynilpyrrolidone (PVP). The homogenate was centrifuge at 12,000 g for 15 minutes and the supernatant was stored at 40 c and later used to determine SOD, POD and CAT. SOD

SOD activity was determined following Zhou et. al., (2003) with slight modification. The reaction mixture containing 81 mL of 14.5 mM methionine, 3 mL of 2.25 mMNitrobluetetrazolium chloride (NBT), 3 mL of 3 μM EDTA-Na_2 and 3 mL of 60 μM riboflavin, was prepared with a 0.05 M sodium phosphate buffer (pH 7.8) with the exception of riboflavin which was prepared using deionized water. 50 μL enzyme extract was

added to the tubes containing 3 mL of the reaction mixtures. The mixtures in the tubes were then exposed to illumination incubator for duration of 20 min with one kept in the dark to serve as control. The absorbance was recorded using a spectrophotometer at 560 nm. One unit of SOD enzyme activity was defined as the quantity of SOD required to produce 50% reduction of NBT under experimental conditions and the specific enzyme activity expressed in units per g leaves fresh weight (FW).

POD assay

POD activity was detected following the procedure of Xu et al., (2008), reaction mixtures containing 75 mL of 100 mM sodium phosphate buffer (pH 6.0), 28.5 μL H_2O_2 (30%) and 42 μLguaiacol, were prepared just before use. 1 mL enzyme extract then added to the 3 mL reaction mixtures. The increasing absorbance was measured at 470 nm at 1 min intervals up to 3 min using spectrophotometer. One unit of POD enzyme activity was defined as absorbance changes at 470 nm per minute. Enzyme specific activity was expressed as units per g FW.

CAT assay

CAT activity was determined using a spectrophotometer following Xu et al., 2008, in which the reaction mixture containing 1.5 mL of 0.05 M sodium phosphate buffer (pH 7.8), 1 mL deionized water and 0.3 mL of 0.1 M H_2O_2 was prepared immediately before use, 0.2 mL enzyme extract was then added to the reaction solution. CAT activity was measured by monitoring the decrease in absorbance at 240 nm due to H_2O_2 consumption. One unit of CAT enzyme activity was defined as changes at 240 nm per minute. Enzyme specific activity was expressed as units per g FW.

Lipid peroxidation

Lipid peroxidation was expressed as malondialdehyde (MDA) content in μM per g FW following Zhang et al. (2005) with minor modifications. The fresh leaves were homogenized in 5 mL of 10% trichloroacetic acid (TCA) using a pestle and mortar. The homogenates were centrifuged at 4000 g for 10 min. To each 2 mL aliquot of the supernatant, 2 mL of 0.6% thiobarbituric acid (TBA) in 10% TCA was added. The mixture were heated at 100°C for 30 min and then cooled in an ice bath. The mixture was centrifuged at 10,000 g for 10 min and the absorbance of the supernatant recorded at 532 nm and 450 nm.
Osmolyte contents detection (proline, protein, total sugar, amino acid)

Estimation of free proline

Proline was determined following Bates et al., 0.3 g of plant sample was homogenized in a 10 ml of 3 % aqueous sulphosalicylic acid. The homogenate was filtered using Whatmann No. 42 filter paper. 2ml of ninhydrin (1.25 g ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid) and 2 ml of glacial acetic acid in a test tube was heated for 30 min at 1000C. The reaction mixture was then extracted with 4 ml toluene and mixed vigorously by using a vortex mixture for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase. The absorbance of the toluene layer was measured in a spectrophotometer at 520 nm using toluene as blank.

Estimation of protein

Protein content was determined following Lowry et al., (1951) with minor modifications. 0.3 g fresh samples were homogenized in 20% trichloroacetic acid using mortar and pestle. The homogenate was then centrifuged at 1200 rpm for 15 min and the supernatant was discarded. Five ml of 0.1 N NaOH was added to the pellet and it was centrifuged for 10 min. The supernatant was saved for the estimation of protein. To 0.5 ml of the extract, 5 ml of copper reagent 'C' was added (Reagent C: mixture of reagents A and B in the 50:1 ratio; Reagent A: 2% $Na_2 CO_3$ in 0.1 N NaOH; Reagent B: equal volume of 1% $CuSO_4$ and 2 per cent sodium potassium tartrate). The tubes were shaken well and allowed to stand in dark for 10 min at room temperature, 0.5 ml of well diluted Folin-Ciocalteau reagent was added to the solution and mixed thoroughly. The absorbance was then taken at 500 nm in a photo spectrophotometer against an appropriate blank. Bovin serum albumin was used as the standard.

Estimation of total sugars

Total sugars were determined following Nelson's (1944) procedure. The process involves treating plant samples with 80 % boiling ethanol for extraction, one ml of ethanol extract in the test tubes was evaporated in water bath. 1 ml of distilled water and 1 ml of 1 N sulphuric acid were then added and incubated at 49 ^{0}C for up to 30 min. The solution was neutralised with 1 N sodium hydroxide using methyl red indicator. One ml of Nelson's reagent was added to each test tube prepared by mixing reagent A and reagent B in 25:1 ratio (Reagent A: 25 g sodium carbonate, 25 g sodium potassium tartarate, 20 g sodium bicarbonate and 200 g anhydrous

sodium sulphate in 1000 ml; Reagent B: 15 g cupric sulphate in 100 ml of distilled water with 2 drops of concentrated sulphuric acid). The test tubes were then heated for 20 min in a boiling water bath, the mixture was then allowed to cooled down and 1 ml of arsenomolybdate reagent (25 g ammonium molybdate, 21 ml concentrated sulphuric acid, 5 g sodium arsenate dissolved in 475 ml of distilled water and incubated at 37^0C in a water bath for 48 h) was added. The solution was thoroughly mixed and diluted to 25 ml and measured at 495 nm in a spectrophotometer. The reducing sugar contents of unknown samples were then calculated from glucose standard.

Estimation of amino acids

Amino acids were determined following Moore and Stein (1948), 1 ml ethanol extract was taken in 25 ml test tubes and neutralized with 0.1 N sodium hydroxide using methyl red indicator. 1 ml of ninhydrin reagent was added (800 mg stannous chloride in 500 ml citrate buffer, pH 5.0, 20 g ninhydrin in 500 ml methyl cellosolve,both solutions were mixed). The mixture was boiled in a water bath for 20 min, 5 ml of diluent solution (distilled water and n- propanol mixed in equal volume) was added, then cooled down and diluted to 25 ml with distilled water. The absorbance was measured at 570 nm using photo spectrophotometer. The standard graph was prepared using leucine.
Growth parameters detection (shoot length, root length, dry matter content)
Morphological parameters including root length, shoot length and dry matter contents per plant were determined for every treatment. Plant and root heights were measured using a ruler at harvest after 45 days growth period. Whereas, dry matter contents was determined by weighing 1hill of oven dried rice samples and results expressed in g/hill.

Cd uptake detection

After oven drying, plant samples were then ground to powdered form and digested using concentrated HNO_3 and $HClO_4$ in an 8:2, V/V as suggested by Allen (1989). After digestion, Cd concentrations in the roots and straw were analysed using a flame atomic absorption spectrometer.

Statistical analysis

Data was analysed using Microsoft excel 2010 and Analysis of variance was performed using Statistix 8 (Analytical, Tallahassee, Florida, USA). The data were analysed by one-way analysis of variance to assess differences in parameters between treatments.

III. Results

Cadmium effects on biochemical content in rice leaves

Proline, protein, amino acid and total sugar contents in rice leaves were determined and results presented in table 1. The contents of proline, protein, amino acid and total sugar were all found reducing with increased Cd toxic levels in the soil. There were significant differences in all the treatments for all cultivars. The leaves proline, protein, amino acid and total sugar contents were found maximum at the control treatment (0 mg kg $^{-1}$) for all the cultivars, while the minimum contents were recorded at the 100 mg kg $^{-1}$ soil level treatments for all cultivars. Guixiangzhan showed the highest proline, protein, amino acid and total sugar contents followed by Meixiangzhan 2 and the least on Xiangyaxiangzhan. There were34.68 and 62.68 % for Meixiangzhan 2, 19.32 and 57.895% for Xiangyaxiangzhan and 14.81 and 39.81%decreasesfor Guixiangzhanin prolinecontents for both 50 and 100mg Cd/kg soil respectively when compared to the control treatment. For protein contents, the decreases were; 3.27 and 21.36% in Meixiangzhan 2, 27.85 and 40.32% in Xiangyaxiangzhan and 0.71 and 6.42% in Guixiangzhan for both 50 and 100mg Cd/kg soil respectively when compared to the control treatment. For amino acid contents, there were 34.81 and 47.84% in Meixiangzhan 2, 38.52 and 61.59% in Xiangyaxiangzhan and 18.76, and 24.95% in Guixiangzhan decreases for both 50 and 100mg Cd/kg soil respectively when compared to the control treatment. For total sugar contents, the decreases were 13.14 and 36.83% in Meixiangzhan 2, 8.22 and 36.46% in Xiangyaxiangzhan and 10.43 and 19.39% in Guixiangzhan for both 50 and 100mg Cd/kg soil respectively when compared to the control treatment. This shows the extent of cultivar variation in response to rice biochemical contents under Cd toxicity.

Table 1. Cadmium effects on biochemical content in rice leaves

Variety	Treatments	Proline content (µg/g)	Protein content(µg/g)	Amino acid content (mg/kg)	Total sugar content (mg/kg)
Meixiangzhan 2	Cd0	25.0a	479.00a	7.67a	25.33a
	Cd1	16.33b	463.33a	5.00b	22.00b

	Cd2	9.33c	376.67b	4.00b	16.00c
Xiangyaxiangzhan	Cd0	19.0a	403.33a	8.67a	28.33a
	Cd1	15.33b	291.00b	5.33b	26.00b
	Cd2	8.00 c	240.67c	3.33c	18.00c
Guixiangzhan	Cd0	27.0a	490.50a	7.33a	22.33a
	Cd1	23.0ab	487.00a	6.33ab	20.00ab
	Cd2	16.25b	459.00b	5.00b	18.00 b

Means having different letters indicate significant differences at p < 0.05.

Cadmium effects on changes in antioxidant enzymes (SOD, POD, CAT) and MDA in rice roots and shoots

Changes in the activities of SOD, POD, CAT and MDA were observed in both roots and leaves for all treatments in the different rice cultivars and results presented in tables 2. The results showed an increase in the activity of SOD with increased levels of Cd concentrations when compared with control for all cultivars. In every increased Cd level, SOD activity was higher compared to control. For Meixiangzhan 2 cultivar, when rice was subjected to 50 and 100 mg/kg Cd toxicity, there was a steadily increase in SOD activities of 9.39 and 25.77 for roots, and 4.75and13.04 for shoots respectively. For Xiangyaxiangzhan cultivar, the increase was 16.31 and28.04for roots and 15.85 and 27.75 for shoots respectively. While for Guixiangzhan cultivar, there was a 23.71 and 33.56 for roots and 30.52 and 40.27for shoots increase respectively.

POD activity for each treatment in all cultivars were determined in both roots and leaves, the results showed a decrease in the activity of POD with increased levels of Cd concentrations when compared with control. In every increased Cd level, POD activity was lower compared to control. For Meixiangzhan 2 cultivar, when rice was subjected to 50 and 100 mg/kg Cd toxicity, there was a steadily decrease in POD activities of 11.30 and 24.67% for roots and,12.16 and 19.36% for leaves respectively. For Xiangyaxiangzhan cultivar, the decrease was 7.06 and21.54% for roots and,0.38 and 15.82% for shoots respectively. While for Guixiangzhan cultivar, there was a 4.17 and16.54 for roots and, 7.83 and 19.03for shoots decrease respectively.

Significant increases were observed in CAT activity with increased Cd toxicity, but at certain toxic level, CAT activities were found reducing. When the increasing CAT activity was compared with Control for all cultivars, CAT activity in Meixiangzhan 2 cultivar subjected to 50 and 100 mg/kg Cd toxicity saw a steadily increase of36.71 and30.81 for roots, and 44.47 and36.12 for shoots respectively. For Xiangyaxiangzhan cultivar, the increase was 10.45 and31.83% and, 22.84 and27.4 % respectively at 50 mg/kg Cd toxicity and then decreased at 100 mg/kg Cd toxicity. While for Guixiangzhan cultivar, there was a 46.86 and 30.99% and,7.37 and36.65% increase in roots and shoots respectively for the 50 mg/kg Cd toxicity, and then found decreased at 100 mg/kg Cd toxicity. Increased Cd concentrations caused an increased CAT activity.

Increased contents of lipid peroxides observed showed more production of toxic oxygen species. Cd toxicity showed significant increase in MDA production for all cultivars with increased Cd toxicity. In Meixiangzhan 2, Leaves showed higher MDA content compared to roots. In roots, when subjected to 50 and 100 mg/kg Cd toxicity, there was a 37.19 and43.75 increase in roots MDA contents compared to control while in leaves, there was a 29.125 and48.45 increase. In Xiangyaxiangzhan, when subjected to 50 and 100 mg/kg Cd toxicity, there was a 18.74 and27.26 increase in roots MDA contents compared to control, while in leaves, there was a 13.64 and26.7increase. In Guixiangzhan, when subjected to 50 and 100 mg/kg Cd toxicity, there was a 23.78 and8.67 increase in roots MDA contents compared to control, while in leaves, there was a 12.00 and21.93 increase. When rice grows under stressed conditions, free radicals generated in excess do accumulate in the cells, this leads to lipid peroxidation of bio membranes which forms MDA as end product. Hence, increase in MDA concentration is an indicator of physiological stresses and the aging process (Chen et.al. 2003).

Table 2. Cadmium effects on antioxidant enzymes and MDA in rice roots and shoots

Variety	Treatments	SOD (U/gfw)		POD(U/g.min)		CAT(U/(g.min))		MDA(μmol/g)	
Meixiangzhan 2	Cd0	Roots	Stem	Roots	Stem	Roots	stem	Roots	stem
		64.33c	46.67 a	209.33a	74.00 a	235.00 c	137.33 c	9.00 b	5.67 c
	Cd1	71.00 b	49.00 a	185.67b	65.00 b	371.33a	247.33 a	14.33a	8.00 b
	Cd2	86.67 a	53.67 a	157.67c	59.67 c	339.67b	215.00 b	16.00 a	11.00 a
Xiangyaxiangzhan	Cd0	65.00 c	46.00 c	237.50a	86.33 a	237.00 c	168.67 c	8.67c	6.33 b
	Cd1	77.67b	54.67 b	220.67a	86.00 a	379.33a	278.67 a	10.67b	7.33 ab
	Cd2	90.33a	63.67 a	186.33b	72.67 a	347.67b	232.33 b	14.67a	10.00 a
Guixiangzhan	Cd0	64.33c	44.00 c	231.67a	89.33a	127.00 b	129.67 b	10.67b	7.33 b
	Cd1	84.33b	63.33 b	222b	82.33 a	239.00 a	140.00 b	14.00 a	8.33 b
	Cd2	97.00 a	73.67 a	193.33c	72.33 b	346.33a	221.00 a	15.33a	10.67 a

Means having different letters indicate significant differences at *p* < 0.05

Cd concentration in rice roots and straw at harvest (ug/g)

Cd uptake and accumulation in various plants parts (roots and shoots) of the three rice genotypes used in the experiment were detected and results shown in Table 3. In all cultivars, Cd accumulation in the various organs (roots and shoots) was found elevated with increased concentrations of soil Cd contents. In average, Cd

accumulation was in the descending order: root>straw for all the Cd treatments in all cultivars. The concentration of Cd in various rice organs (root>straw) were significantly different among the three aromatic rice varieties as shown in table 3 above. In terms of uptake, there were outstanding differences in Cd uptake between the different rice organs especially from roots to stems, Guixiangzhan showed the least uptake in both roots and shoot for all treatments followed by Xiangyaxiangzhan while Meixiangzhan showed the highest uptake. Variation in transfer factors from roots to shoots were also observed and were found maximum in Meixiangzhan followed by Xiangyaxiangzhan while Guixiangzhan showed the least for all treatments.

Table 3 CdUptake in Rice Roots and Straw at Harvest (ug/g)

Variety	Treatments	Roots	Straw	whole plant	R-S Transfer factor
Meixiangzhan 2	Cd0	8.25c	1.98c	10.23	0.24
	Cd1	197.01b	11.09b	208.09	0.056
	Cd2	241.40a	16.74a	258.139	0.069
Xiangyaxiangzhan	Cd0	5.66c	1.84c	7.5	0.32
	Cd1	141.89b	10.02b	151.91	0.07
	Cd2	185.21a	16.55a	201.76	0.09
Guixiangzhan	Cd0	4.52c	1.79c	6.31	0.39
	Cd1	84.93b	6.61b	91.54	0.07
	Cd2	117.85a	9.46a	127.31	0.08

Means having different letters indicate significant differences at $p < 0.05$

Growth Parameters

Rice plants height, shoots and roots dry weight for the different treatments in all cultivars were determined after the rice plants were all harvest (after 45 days vegetative growth period) and the results presented in table 4. Rice plants height, shoot and root dry weights were maximum at control treatments for all cultivars and Guixiangzhanshowed the highest height and dry weights contents, while the minimum plants height, root and shoot dry weight was recorded at 100 mg kg $^{-1}$ cadmium levels for all the cultivars and Meixiangzhan 2 showed the least in terms of height and dry weights. Maximum to minimum plants height, shoots and roots dry weight for the different treatments in all cultivars were in the order Guixiangzhan>Xiangyaxiangzhan>Meixiangzhan 2.

Table 4. Growth parameters

Variety	Treatment	plant height (cm plant^{-1})	SHOOT DRY WEIGHT (g/hill)	ROOTS DRY WEIGHT (g/hill)
Meixiangzhan 2	Cd0	38.67a	3.00a	1.50a
	Cd1	35.33b	2.50b	1.30a
	Cd2	32.00c	1.73c	0.97b
Xiangyaxiangzhan	Cd0	41.00a	3.27a	1.57a
	Cd1	36.00b	2.73b	1.10b
	Cd2	34.00c	1.50c	0.97b
GUIXIANGZHAN	Cd0	47.33a	3.80a	1.77a
	Cd1	46.67a	3.63a	1.73a
	Cd2	44.67b	3.30b	1.50b

Means having different letters indicate significant differences at $p < 0.05$

IV. Discussions

Antioxidants enzymes play a very vital role in plants defence mechanisms. Abiotic stresses like heavy metals especially Cd results to molecular damage in rice plant cells due to the generation of reactive oxygen species (ROS) (Hegedus., 2004). Even though Cd by itself does not directly generate ROS, but rather generates oxidative stress by interrupting essential antioxidant defencesystem. Antioxidant enzymes balance the production and destruction of ROS. In our experiment, the activities of catalase, peroxidase and superoxide dismutase were measured as antioxidant enzymes and the results showed a significant different in the means of the different treatments for all cultivars. Our study revealed that the activities of SOD in the root and straw for all the cultivars increased at low soil Cd levels (Table 2). The increase in SOD activity can be attribute to Cd-induced phytotoxicity as concluded by Hou and Kao(2007), this acts as an adaptive response means of plants exposed to heavy metals toxicity (Camp, 1996). However, our findings reveal that SOD activity decreased with increased soil Cd level, suggesting that the high soil Cd level might have damaged the antioxidant defencesystem by reducing SOD activity in the rice roots and straw. The decline in POD and CAT activities with increased soil Cd levels was observed in all cultivars. It revealed that antioxidant enzymes like SOD, POD and CAT work in coordination to minimize oxidative stress in rice plants during growth. In summary, the activities of SOD, POD and CAT in both roots and straw showed significant differences between rice treatments as well as cultivars after exposure to Cd toxicity.

Oxidative stress in rice plant as a result of contamination by toxic metals can be manifested in the activity of MDA, which is a significant indicator of lipid peroxidation (Chaouiet al., 1997). Results from our study showed significant increases in MDA contents in all cultivars after exposed to Cd toxicity (Table 2), this increase indicated toxic effects in rice roots and straw.

Less changes in MDA content was observed in Guixiangzhan and Xiangyaxiangzhan when compared to the other two varieties subjected under the same Cd treatment. This indicates that lipid peroxidation was higher in Meixiangzhan 2 than that in Guixiangzhan and Xiangyaxiangzhan,this is possibly due to the higher uptake ability of Cd in Meixiangzhan 2 cultivar compared to Guixiangzhan and Xiangyaxiangzhan. Decline in biochemical content (Proline, protein, amino acid and total sugar)with increased Cd levels also suggested a strong induction of oxidative stress.

Cadmium concentrations in straw and root of all cultivars significantly increased withincreased levels (Table). Cadmium contents in roots and strawwere higher in Meixiangzhan 2 than that in Guixiangzhan and Xiangyaxiangzhan for all treatments, this wasprobably as a result of the higher uptake and translocation ability of Cd from the soil medium to roots and then to straw. The differences observed in accumulation may be related to the genotypic tolerance nature of the cultivars to cadmium toxicity. Genotypic variations of Cd tolerance in rice cultivars were reported by Liu et al. (2005). Cadmium uptake and translocation from contaminated soil to plants edible parts were markedly different among plant species as well as cultivars within the same species (Liu et al. 2007). In our study, differences in Cd uptake between cultivars were observed, this might be as a result of roots oxidation ability, roots organic acid secretions and roots acidification (Liu et al. 2007).

V. Conclusions

Result from this study has shown that, cadmium toxicity in growing rice seedlings impeded morpho-physiological and antioxidants activities in rice during early growth. Cd toxicity damages the antioxidant defence system resulting to oxidative stress in organs of growing rice plants, this resulted to alteration inAntioxidant enzymes (Superoxide dismutase (SOD), Peroxidase(POD), and Catalase(CAT)), osmolyte accumulation (proline, protein, total sugar, amino acid), lipid peroxidation (in terms of melanodialdehydeMDA) and growth parameters (plant height, and total dry matter content). At 100 mg/kg soil Cd toxicity, growing rice seedlings become critical and vulnerable to toxicity. The results suggested that soil Cd toxicity have negative consequences on rice growth. Rice roots accumulated higher Cd than shoots and uptake varies amongst cultivars. Conclusively, Cd toxicity impaired early growth in rice by affecting physio-biochemical attributes, however, Guixiangzhan variety performed better than the other two varieties.

Acknowledgement

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