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CADMIUM TOXICITY INDUCED MORPHOLOGICAL CHANGES DURING GERMINATION AND SEEDLINGS IN TWO RICE *CVS.* DR-92 AND BH-1 FROM NORTH EAST INDIA

Sareeta Nahakpam
Department of Biochemistry and Crop Physiology, Bihar Agricultural University, Sabour,
Bhagalpur-813210, Bihar, India
E-mail: nichsareeta@gmail.com

Abstract: A 100 percent germination of seeds by 96 h of germination in both the rice cultivars DR-92 and Bh-1 were observed, that decreased under Cd^{2+} treatments. Cd^{2+} concentrations of 10, 50, 100 and 500 µM caused a significant change in the growth parameters with visual symptoms as yellowing of leaves (chlorosis and necrosis) and stunted root growth with extensive browning and thickening of the roots in a concentration dependent manner. The plant biomass of the two rice cultivars increased by 2-2.5 folds at day 10 as compared with that at day 5, the increase being ~ 58 percent under 500 µM Cd-treatments in rice seedlings. At day 20 however, a 50-51 percent increase in biomass were observed. A 50 µM Cd-treatment significantly decreased the plant biomass by 47 percent in *cv*. Bh-1 and a 100 µM Cd and 500 µM Cd-treatments caused a 55 and 47 percent decrease in biomass at day 10 when compared with controls. Uptake of Cd^{2+} increased with increasing days of growth and cadmium concentrations in both the *cvs*. DR-92 and Bh-1. Rice seedlings grown under 500 µM Cd^{2+} had 44.2 µg Cd^{2+} g^{-1} dry wt in *cv*. DR-92 and 30 µg Cd^{2+} g^{-1} dry wt in *cv*. Bh-1 showing more Cd^{2+} uptake in the former than in latter. Throughout the growth period and under all Cd^{2+} levels the uptake/amount of Cd^{2+} were higher in roots than shoots of both the rice cultivars suggesting more accumulation and less translocation of Cd^{2+} from roots to shoots in the growing seedlings.

Keywords: Cadmium, chlorosis, rice, stunted root.

Introduction

Cadmium (Cd^{2+}) is a toxic, non-essential heavy metal with long biological persistence (Wagner, 1993). It causes phytotoxicity and its uptake and accumulation in plants move it further into food chain, leading to a potential threat to human health (Shah and Dubey, 1998). Cadmium is released into the environment by power stations, industrial processes, phosphate fertilizers, nickel-cadmium batteries, gas exhausts from automobiles, etc. (Wagner, 1993; Jiang and Wang, 2007). Non polluted soil solutions contain Cd^{2+} concentrations ranging from 0.04 to 0.32 µM however, in heavily polluted soil it reaches 35 µM and may even exceed 100 µM concentrations (Sanita de Toppi and Gabbrielli, 1999). Cd^{2+} makes one of the most hazardous heavy metals with a high potential toxicity for all kinds of organisms (Jiang and Wang 2007). Although not essential for plant growth, Cd^{2+} ions are readily taken up by roots

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and translocated to above ground parts actively (Shamsi *et al.*, 2008). Several studies demonstrated that cadmium ions can function as stressors, causing some physiological constraints that decrease plant vigor and inhibit plant growth (Nahkapam and Shah, 2011; Schutzendubel *et al.,* 2001). Cd^{2+} also caused various phytotoxic symptoms including chlorosis, inhibition of seed germination, growth inhibition, water imbalance, phosphorus and nitrogen deficiency, reduced manganese transport and accelerated senescence (Shah *et al.,* 2013; Sanita de Toppi and Gabbrielli, 1999; Benavides *et al.*, 2005). The presence of Cd^{2+} has also been associated with the appearance of oxidative stress (Nahakpam and Shah, 2011; Baryla *et al.*, 2001). Also recently it was reported that Cd^{2+} causes a series of three waves of generation of active oxygen species, where firstly NADPH oxidase-dependent accumulation of hydrogen peroxide, followed by the accumulation of superoxide anion in mitochondria and finally fatty acid hydroperoxides were detected in tobacco cells (Garnier *et al.*, 2006). Access to heavy metals in bare roots is confined to the first few millimeters of the root tip. Toxic effects are exerted to the plasma membrane within the cell (Arduini *et al.*, 1996). Cd^{2+} when taken up by the roots enters it and is loaded into the xylem through an apoplastic and/or a symplastic pathway for its transportation into leaves (Rodriguez-Serrano *et al.*, 2006). Normally, Cd^{2+} ions are mainly retained in the roots and only small amounts are transported to the shoots (Cataldo *et al.,* 1983). Such accumulation and translocation of Cd^{2+} in roots to leaves differs considerably among species and/or even among varieties of the same species. Mostly Cd^{2+} gets deposited and binds largely in the cell walls adjacent to plasma membrane and to the endomembrane compartments (Kocjan *et al.*, 1996), however, in leaves Cd^{2+} are found to accumulate in vacuoles as well.

The increase in vacuolation was the first visible effect of metal toxicity on the meristematic cells treated with 1mM Cd^{2+} and presence of electron dense granules were also reported between the cell wall and plasmalemma in roots of Allium cepa (Liu and Kottke, 2004). At high concentration of Cd^{2+} the cell is damaged leading to condensed cytoplasm, reduction in the number of mitochondrial cristae, severe plasmolysis and decrease in number of ribosomes. These structural alterations ultimately lead to death of most cells (Liu and Kottke, 2004). The present study was therefore undertaken to examine the effects of increasing levels of Cd^{2+} toxicity on the germination percent and seedling vigour of two rice cultivars DR-92 and Bh-1. An attempt has been made to examine the uptake, translocation, localization and accumulation of Cd^{2+} in rice tissues.

Materials and Methods

Plant material and stress conditions

Two rice (Oryza sativa) cultivars, cv. DR-92 and cv. Bh-1 from North East India were used. Seeds were germinated in petriplates for 5 days at 28±1°C with 80 percent relative humidity and in a regular cycle of 12 h light followed by dark period. Seeds were germinated in petriplates using distilled water as control or by adding 10 µM, 50 µM, 100 µM and 500 µM $Cd(NO_3)_2$ solutions.

After 5 days of germination the rice seedlings were raised in sand cultures, saturated either with Hoagland nutrient solution (Hoagland and Arnon, 1938) that served as control or nutrient solution supplemented with 10 µM, 50 µM, 100 µM and 500 µM $Cd(NO_3)_2$ as treatments. Cd^{2+} levels were ascertained as low toxic (10 µM, 50 µM) and high toxic (100 µM, 500 µM) concentrations with full viability of seeds. Seedlings were maintained in the growth chamber for 20 day at 28±1^{o}C, 80 percent relative humidity and 12-h light/dark cycle as described above. Seedlings were uprooted at 5 day intervals and roots and shoots were separated which served as Cd^{2+} treated plant samples. The roots and shoots were separated and all the experiments were performed in triplicate.

Evaluation of germination percent and calculation of plant biomass

Germination percent was evaluated using 50 healthy seeds from each of the two rice cultivars in triplicate. Completely germinated seeds were counted at 24 h intervals at 0, 24, 48, 72, 96 and 120 h of germination. To evaluate plant biomass seedlings were oven dried and is calculated as g^{-1} dry weight.

Extraction and estimation of Cd^{2+} in growing rice seedlings

Oven dried seedlings were used for the estimation of Cd^{2+}. The samples were initially ground to a fine powder and then digested in concentrated HNO_3 followed by its dissolution in dilute perchloric acid (PCA) which led to the release of bound Cd^{2+} in ionic form in the solution. Cd^{2+} content measured using Atomic Absorption Spectrophotometer (AAS) and expressed in terms of µg g-1 dry wt.

Statistical analyses

The experimental values obtained in the present study are mean ± SD. based on three replicates of three independent experiments. The significant differences were assessed by the analysis of variance (ANOVA) test, taking $P<0.05$ as significant according to Turkey's multiple range.

Results

Common whole plant responses of cadmium on germination and biomass in rice have been discussed in the result. A 100 percent germination of seeds in controls by 96 h of germination in both the rice cultivars was observed (data not shown). Inspite of a decrease in germination percentage under Cd^{2+} treatments as compared to controls, our result showed 100 percent germination in both the rice cultivars at 120 h under 500 µM Cd^{2+} levels, suggesting full viability of the seedlings.

Table 1. Visible injuries in shoots and roots of rice seedlings grown under increasing cadmium concentrations (10, 50, 100, 500 µM) of $Cd(NO_3)_2$ at 5 and 15 days of growth

Cd exposure (Days)	Cd concentrations (µM)	Shoot symptoms (chlorosis and necrosis)	Root symptoms (browning/stunting)
5	0	*	*
	10	*	+
	50	+	+
	100	++	+
	500	++	+
15	0	*	*
	10	+	+
	50	+++	++
	100	+++	+++
	500	++++	++++

* absent, + very slight, ++ mild, +++ moderate, ++++ severe

The application of Cd^{2+} at a concentration of 10, 50, 100 and 500 µM caused a significant change in the growth parameters with visual symptoms as yellowing of leaves (chlorosis and necrosis) and stunted root growth with extensive browning and thickening of the roots in a concentration dependent manner (Table 1).

Table 2 shows that the plant biomass in g fresh wt in control plants of the two rice cultivars increased by 2-2.5 folds at day 10 as compared with that at day 5. A 50 µM Cd-treatment significantly decreased the plant biomass by 47 percent in cv. Bh-1 at day 10 of the growth period than that in control.

Table 2. Effect of increasing concentrations of (10, 50, 100, 500 µM) cadmium on plant biomass in rice *cvs.* DR-92 and Bh-1. The values are mean of triplicates ± SD. ANOVA significant at P≤0.01. Values with different letters are significantly different at P< 0.05.

Stress treatments	**Biomass (g^{-1} fresh wt)**							
	***cv.* DR-92**				***cv.* Bh-1**			
	DAYS OF GROWTH							
	5	**10**	**15**	**20**	**5**	**10**	**15**	**20**
Control	0.24^{a}	0.64^{b}	11.1^{c}	12.1^{a}	0.36^{a}	0.72^{b}	15.6c	15.1^{c}
10 µM Cd	0.22^{a}	0.60^{b}	10.2^{c}	10.0^{c}	0.29^{a}	0.62^{b}	14.8^{c}	14.9^{c}
50 µM Cd	0.24^{a}	0.62^{b}	14.6^{c}	9.99^{b}	0.30^{a}	0.43^{a}	14.1^{c}	13.8^{c}
100 µM Cd	0.23^{a}	0.59^{b}	9.84^{b}	9.32^{b}	0.23^{a}	0.40^{a}	12.2^{c}	13.6^{c}
500 µM Cd	0.19^{a}	0.63^{b}	13.4^{c}	1.04^{c}	0.21^{a}	0.34^{a}	11.5^{c}	12.2^{c}

However, under 100 µM Cd and 500 µMCd-treatments a 55 percent and 47 percent decrease in biomass respectively were noted at day 10 when compared with controls. No significant changes were observed in cv. DR-92 under all the Cd^{2+} treatments. At day 5, in controls and ~ 58 percent increase in biomass under 500 µM Cd-treatments in rice seedlings of the cvs. DR-92 and Bh-1 were noted. At day 20 however, a 50-51 percent increase in biomass is observed in the two cultivars, under similar stress conditions.

The presence and accumulation of Cd^{2+} in the root were almost similar with no significant variations in the two rice cvs. DR-92 and Bh-1 except that more Cd^{2+} uptake was in former than in latter. The results therefore, further strengthen the observation that there is higher uptake of Cd^{2+} under high Cd^{2+} levels in roots of cv. Bh-1 than in cv. DR-92, as compared to controls. Least accumulation of Cd^{2+} in the shoot part of the seedlings revealed the higher absortption and accumulation potential of roots than that of shoots (Fig. 1). Moreover, result further suggested a least translocation of accumulated Cd^{2+} from root to shoot at early stress exposure in all the Cd^{2+} treatments.

Staining of 15 day old root tips of rice seedlings cv. DR-92 exposed to 500 µM Cd^{2+} (data not shown) brought about a Cd-specific stress-induced morphogenic response (SIMR) phenotype, characterized by an inhibition of root elongation and enhanced formation of lateral roots.

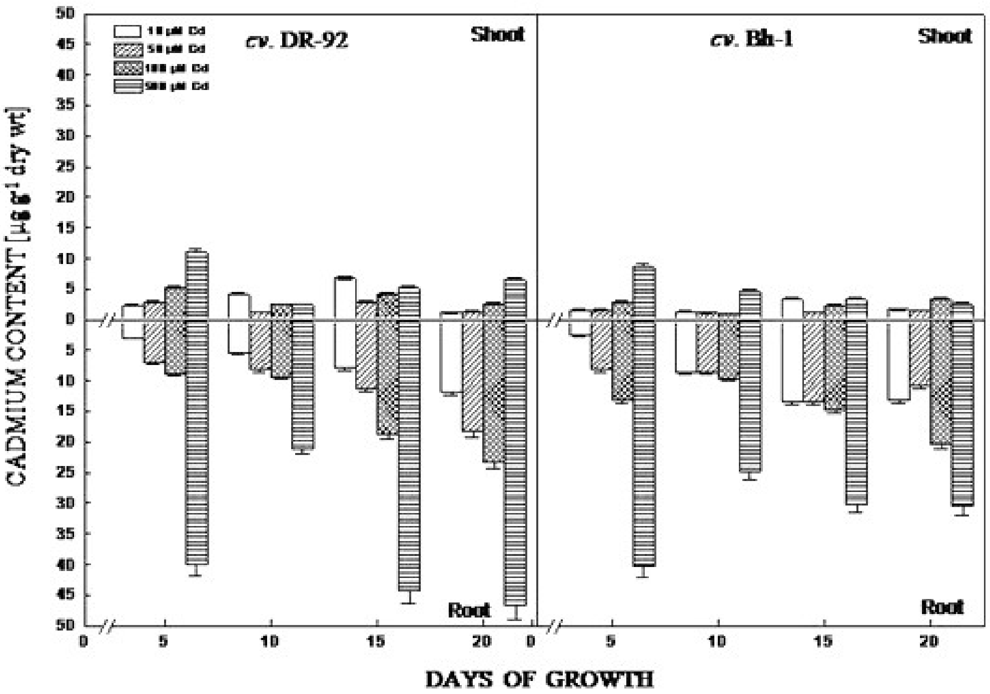

Fig 1. Cadmium content in the root and shoot tissues of rice *cvs*. DR-92 and Bh-1 at increasing days of growth under increasing concentrations of cadmium in the growth medium. Values are mean of three replicates ± SD

Discussion

Although non-essential for plant growth, Cd^{2+} ions are readily taken up by the roots and translocated to above-ground parts causing several physiological disorders like inhibition of seed germination and reduction of plant growth (Sanita de Toppi and Gabrielli 1999). The results showed a 100 percent germination of seeds in controls by 96 h of germination. At 120 h ~ 100 percent germination were noted under 500 µM Cd(NO$_3$)$_2$ levels. There are similar reports in apricot (Gur and Topdemir, 2008) and sunflower (Hatata and Abdel-Aal, 2008).

High soil Cd^{2+} levels resulted in its increased uptake and accumulation in rice roots (Shah and Dubey 1998). An accumulation of as high as 44 µg g-1 dry wt Cd^{2+} and 5.12 µg g-^{1} dry wt Cd^{2+} under 500 µM Cd^{2+} levels at day 15 roots and shoots of cv. DR-92, respectively as noted in this work suggests more uptake and accumulation of Cd^{2+} by roots with a less translocation to shoots.

There was an obvious difference between the growth parameters of the two rice genotypes. The genotypic differences observed in the distribution of Cd^{2+} in plants observed in the shoot and root Cd^{2+} content has been shown to be a result of differences in internal distribution rather than Cd^{2+} uptake. Above a certain critical root Cd^{2+} concentration, specific for a given inbred lines, the roots became unable to retain more Cd^{2+} (Florijn and van Beusichem, 1993).

The extent of Cd^{2+} accumulation is also shown to be related to age of the plant. In maize leaves treated with 200 µM Cd^{2+}, the Cd^{2+} content increased in parallel with age (Drazkiewicz *et al.,* 2003).

Conclusion

The results of this study therefore lead to the conclusion that Cd^{2+} toxicity exerts phytotoxic symptoms in rice that appears as visible plant injuries as stunted root growth, discolored leaves, thickening, hardening and browning of roots. A decrease in root and shoot lengths is also observed. A higher Cd^{2+} uptake occurs in root elongation zone. Varied growth parameters for the two rice cvs. DR-92 and Bh-1 are evident. Rice cv. Bh-1 accumulates more Cd^{2+} in roots with lower translocation to shoots. The result also revealed that Cd^{2+} primarily accumulated in vacuoles.

References

[1] Arduini I, Godbold D L, Onnis A. 1996. Cadmium and copper uptake and distribution in Mediterranean tree seedlings. Physiol. Plant. 97:111-117.

[2] Baryla A, Carrier P, Franck F, Coulomb C, Sahut C, Havaux M. 2001. Leaf chlorosis in oilseed rape plants (Brassica napus) grown on cadmium-polluted soil: causes and consequences for photosynthesis and growth. Planta 212:696-709.

[3] Benavides M P, Gallego S M, Tomaro M L. 2005. Cadmium toxicity in plants, Braz. J. Plant Physiol. 17: 21-34.

[4] Cataldo C D, Garland T R, Wildung R E. 1983. Cadmium uptake, kinetics in intact soybean plants. Plant Physiol. 73:844-848.

[5] Drazkiewicz M, Tukendorf A, Baszynski T. 2003. Age-dependent response of maize leaf segments to cadmium treatment: effect on chlorophyll fluorescence and phytochelatin accumulation. J Plant Physiol. 160: 247-254.

[6] Florijn P J, Van Beusichem M L. 1993. Cadmium distribution in maize inbred lines: Effects of pH and level of Cd supply. Plant and Soil 153(1): 79-84.

[7] Garnier L, Simon-Plas F, Thuleau P, Angel J, Blein J, Ranjeva R, Montillet J. 2006. Cadmium affects tobacco cells by a series of three waves of reactive oxygen species that contribute to cytotoxicity. Plant Cell Environ. 29:1956-1969.

[8] Gür N, Topdemir A. 2008. Effects of some heavy metals on in vitro pollen germination and tube growth of apricot (Armenica vulgaris Lam.) and cherry (*Cerasus avium* L.). World App. Sci. J. 4(2):195-198.

[9] Hatata MM, Abdel-Aal EA. 2008. Oxidative stress and antioxidant defense mechanisms in response to cadmium treatments. American-Eurasian J. Agric. and Environ. Sci 4(6): 655-669.

[10] Hoagland D R, Arnon D I. 1938. The water culture method for growing plants without soil. Cal. Agri. Exp. Sta. Cir. 3: 346-347.

[11] Jiang X, Wang C. 2007. Cadmium distribution and its effects on molybdate-containing hydroxylases in Phragmites australis. Aqua. Bot. 86:353-360.

[12] Kocjan G, Samardakiewicz S, Wozny A. 1996. Regions of lead uptake in Lemna minor plants and localization of this metal within selected parts of the root. Biol. Plant. 38:107-117.

[13] Liu D, Kottke I. 2004. Subcellular localization of cadmium in the root cells of *Allium cepa* by electron energy loss spectroscopy and cytochemistry. J Biosci 29(3): 329-335.

[14] Nahakpam S, Shah K. 2011. Expression of key antioxidant enzymes under combined effect of heat and cadmium toxicity in growing rice seedlings. Plant Growth Regul. 63: 23-35.

[15] Rodriguez-Serrano M, Romero-Puertas M C, Zabalza A, Corpas F J, Gomez M, del Rio L A, Sandalio L. 2006. Cadmium effect on oxidative metabolism of pea (Pisum sativum L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo. Plant Cell Environ. 29:1532-1544.

[16] Schutzendubel A, Schwanz P, Teichmann T, Gross K, Langenfeld-Heyser R, Godbold D L, Polle A. 2001. Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in pine (Pinus sylvestris) roots. Plant Physiol 127:887–898.

[17] Shah K, Dubey RS. 1998. Cadmium elevates level of protein, amino acids and alters activity of proteolytic enzymes in germinating rice seeds. Acta Physiol. Plant. 20:189-196.

[18] Shah K, Singh P, Nahakpam S. 2013. Effect of cadmium uptake and heat stress on root ultrastructure, membrane damage and antioxidative response in rice seedlings. J. Plant Biochem. Biotech. 22(1):103-112.

[19] Sanita di Toppi L, Gabbrielli R. 1999. Response to cadmium in higher plants, A review. Environ. Expt. Bot. 41:105-130.

[20] Shamsi I H, Wei K, Zhang G P, Jilani GH, Hassan M J. 2008. Interactive effects of cadmium and aluminum on growth and antioxidative enzymes in soybean. Biol. Plant. 52(1):165-169.

[21] Wagner G J. 1993. Accumulation of cadmium in crop plants and its consequences to human health. Adv. Agron. 51:173-212.