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Genotypic response of high-yielding lowland rice varieties of Odisha to cadmium

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ABSTRACT

Cadmium contamination in agricultural soils is of global concern because its concentration in soils is enhanced now-a-day due to application of pesticides and phosphatic fertilizers. Three high yielding low land rice varieties of Odisha, var. Hiranmayee, var. Mrunalini and var. Jagannath, were investigated for their genotypic responses under low doses (0 to 500 µM) of cadmium in hydroponic cultures. Rice varieties were grown in YS nutrient solution with different concentrations of Cd and photosynthetic pigment analysis, heavy metal tolerance indices, phytotoxic symptoms and overall plant growth parameters were evaluated every 5 day interval for 20 days. Chlorophyll (*Chl*) and carotenoids pigment levels significantly decreased over time with increasing Cd concentration. Increased *Chl* a/b ratio indicated a marked fluctuations in the *Chl* b level. Total *Chl* content reduced with a prolongation of treatments, proportionately with the test concentrations. Chlorosis was primarily observed from 14th day of the treatment. Root growth was inhibited with a conspicuous blackening and loss of adventitious rootlets at 200 and 500 µM of Cd^{2+} in the solution, while 10 and 50 µM Cd^{2+} in nutrient solution stimulated the plant growth. Tolerance index values reported in var. Mrunalini to be of higher Cd tolerance while var. Jagannath to be lower Cd tolerance at Cd level below 500 µM.

1. Introduction

Plants are continuously being subjected to biotic and abiotic stress due to diverse anthropogenic activities from various sources like industrial effluents and wastes, urban run-off, sewage treatment plants, agricultural fungicides and mining operations. Such environmental pollutants are progressively affecting the agricultural land and other different ecosystems which largely contribute to soil pollution (Macfarlane and Burchett, 2001) that is not only contaminating the agricultural land, but also incorporating fast into the plant tissue (Berrow and Webber, 1972; Williams and David, 1976). Various abiotic stresses caused due to heavy metals, e.g., aluminium (Al), lead (Pb), and cadmium (Cd), are associated with the growing problems of agricultural soil contamination on a global scale (Azevedo *et al*., 2012). Cadmium contamination in agricultural soils is of particular concern as its supplementation in agricultural soils is enhanced, mainly due to application of pesticides and phosphatic fertilizers. Cadmium is a highly water-soluble heavy metal, easily available in the soil. It is fast uptaken by plants owing to its high mobility in the soil-plant system and easily accumulates in the edible plant parts, causing yield reduction. Consumption of heavy metal contaminated plant products severely affects mammalian tissues including kidneys, liver and lungs (Nakadaira and Nishi, 2003).

Cadmium is a stable element due to its long biological half-life and therefore stress effects of cadmium in the plant system is severe and prolonged. Common visual symptoms include leaf chlorosis, leaf and root necrosis and a prominent growth inhibition and tissue-size (Hernandez and Cooke, 1997). Phytotoxicity of the metal is mainly due to the binding of the metal to the SH-containing proteins, membrane phospholipids and oxidative phosphorylation (Wagner, 1993), leading to an impairment of cell respiration, inhibition of enzyme activities and protein denaturation (Das *et al.*, 1997; Hernandez and Cooke, 1997; Mittra *et al.*, 2009). Several

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methods like chemical remediation and phytoextraction have been proposed to reduce Cd concentration in paddy soil (Ishikawa *et al*., 2006; Makino *et al*., 2008). However, growing concentration of Cd in the soil in contaminated areas is a serious concern for rice yield. Present study aims to investigate the low doses of Cd in three low-land high-yielding rice varieties with regard to their efficacy in cope with the Cd stress and other physiomorphological effects.

2. Materials and methods

2.1. Plant materials

Rice seeds (*Oryzae sativa* L.) of var. Hiranmayee, var. Mrunalini and var. Jagannath were collected from Rice Research Station, Orissa University of Agriculture and Technology, Odisha. The agronomic characteristics of the three varieties showed that var. Hiranmayee had the lowest 135 days crop duration time where as maximum was 150 days in var. Jagannath following by var. Mrunalini (146 days). The average maximum yield was 5654 ka/ha in var. Mrunalini followed by 5453 kg/ha in var. Hiranmayee and 5000 kg/ha in var. Jaganath.

2.2. Chemical treatment

Cadmium available as the hydrated form of cadmium chloride (E-Merck) was prepared as a stock of 1 mM in deionized, double distilled water. Appropriate amounts of the stock solution were diluted in MilliQ water to attain working concentrations (10µM-500µM).

2.3 Seed germination

Seeds were surface sterilized with a 0.5% Bevistin (fungiside) for 15 min and a brief treatment of 70% ethyl alcohol for 3 min followed by an intermittent thorough washing with deionised water. Twenty-five seeds were imbibed in the petri dishes for 12h and incubated in dark for 72h at 37^0C. Germination was considered when the coleoptiles were longer than 2 mm.

2.4 Hydroponic culture

Uniformly germinated seeds were selected and transferred to the plastic cups containing Yoshida's Nutrient solution (Yoshida, 1975) and spiked with different concentrations of Cd^{2+}. Seeds immersed in the nutrient solution without the test solution served as control. Three replicates were carried out for each treatment and the required analysis was performed from the 5^{th} day till 20^{th} day.

2.4 Morphological parameters

Phytotoxic symptoms such as the leaf color and variations in the plant health were analyzed by direct visualization of the samples. Growth parameters such as the length of roots and shoots were measured directly using a ruler at every 5 day interval. Metal Tolerance Index was calculated based on the root length study as per the formula: Metal Tolerance index = [(Maximum root length in Cadmium solution- Maximum root length in Control condition)/ Maximum root length in Control condition] ×100.

2.5. Estimation of photosynthetic pigments

Photosynthetic pigments were extracted from 0.5g of the fresh leaf using 80% acetone as the extracting solution and the absorbance of the extract was read at 646.8 nm, 663.2 nm for chlorophyll and 470 nm for carotenoids. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations (mg/ g) were calculated using the equations given by Arnon (1949).

3. Results and discussion

3.1. Plant growth and morphology

Phytotoxic symptoms were noted at ≥ 100µM of Cd^{2+} in all the three varieties (Figs. 1a & 1b). The concentration of Cd^{2+} at 10 µM rather promoted length of root and shoot growth in all the three test varieties throughout the experiment. In var. Jaganath the shoot length increased at 10 µM. Leaf chlorosis and root length inhibition was observed in all the three varieties at ≥ 100 µM Cd^{2+}, while the severity of the root inhibition was accordingly to increasing Cd concentration (Figs. 2a-c). Root length inhibition was the most rapidly observable parameter under Cd toxicity (Guo and Marschner,1995), already reported in other crops (Cheng and Zhou, 2002; Song *et al.,* 2002; Zhou, 2003). The graphical representation for root length elongation (Figs. 2a-c) was in accordance with the morphological data. Shoot length was affected too as the length of shoot was found decreased with increased concentration of Cd^{2+} in all the varieties. However, shoot growth was drastically decreased in 500 µM of Cd^{2+} in var. Hiranmayee (Fig. 1a) followed by var. Mrunalini (Fig.1b) whereas var. Jagannath showed not such significant changes. Other visual symptoms to Cd stress were development of spiny shoots, root curling and discolouration of root-hairs. The effect of Cd treatment on the variety Jagannath did not show any significant variation in the morphological traits. on the contrary, 10µM Cd treatment in all the three varieties did not show any phytotoxic symptoms. Root and shoot length in all the three varieties progressively reduced with the number of days at 100µM, 200 µM and 500 µM of Cd^{2+} ion supplementation (Figs. 2a-c & 3a-c). Differences in plant growth was the most easily detectable parameter for plants under stress (Bindhu and Bera, 2001). Plant height was significantly reduced at 100 µM and 200µM Cd stress while

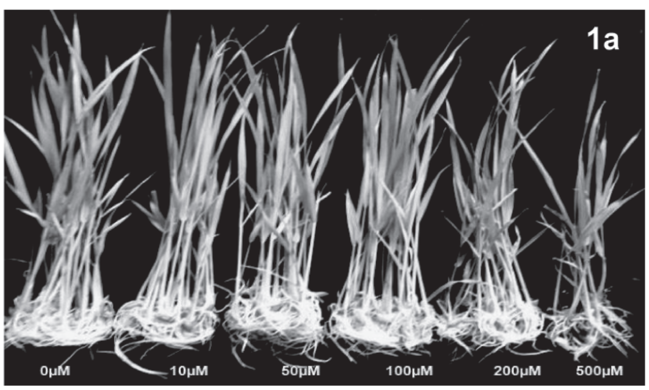
50 µM Cd treatment did not significantly affect the seedlings. Among the varieties it was found that the root of var. Mrulani found significantly susceptible to Cd stress than that of var. Jagannath followed by var. Hiranmayee. In contrast the shoot length was decreased significantly in var. Mrulani with increased Cd concentration (Fig. 1b). Root and shoot length significantly decreased with increase in concentration of Cd. The shoot system was less affected as compared to root at 500µM of Cd.

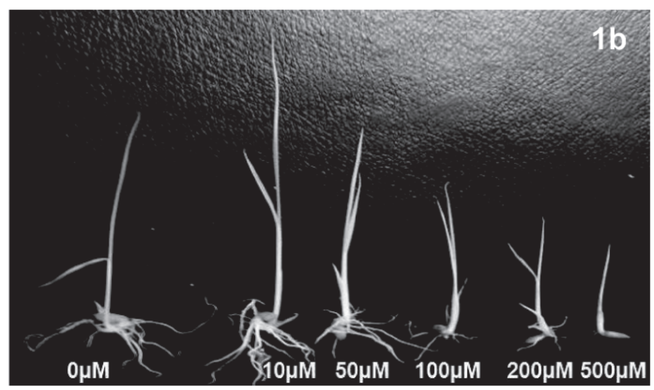
3.2. *Metal tolerance indices*

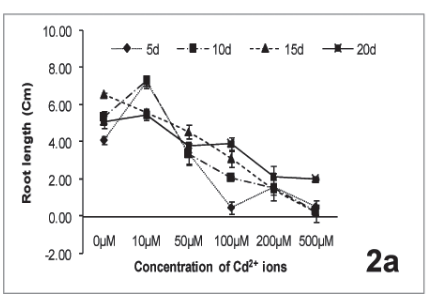
All the three rice varieties varied in their metal tolerance indices (MTI) values (data not shown). Var. Mrunalini was noted to have the highest tolerance index while var. Jagannath showed lower tolerance values consistent at all selected concentrations. MTI for roots could be a measured for the root-to-shoot translocation of the heavy metal ions. Higher values for root MTI might be a defense strategy adopted by the plant species to protect the system from the deleterious effects of elevated Cd levels. Tolerance values in var. Hiranmayee was noted to be higher at 500 µM Cd level, which might be a mechanism to adopt at high abiotic stress. Such tolerance indices may vary with plant species and genotypes based on the rate of Cd intake and its subsequent translocation (Metwally *et. al.,* 2005; Salt *et. al.,* 1995).

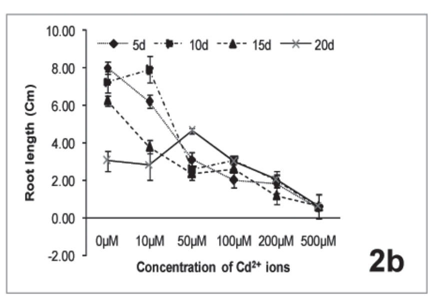
3.3 *Total photosynthetic pigments*

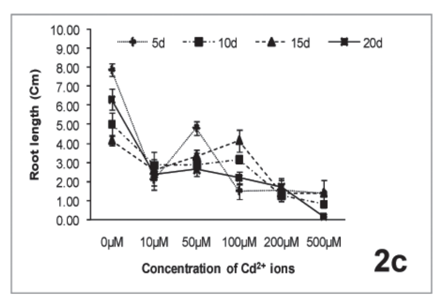
Lowering in the total chlorophyll levels was noted in all the three varieties while carotenoid content tend to increase as a means to counter act the cadmium stress effects on the total chlorophyll content (Fig 3a-c). Chlorophyll *b* was largely affected than chlorophyll *a* at 200µM and 500 µM Cd doses. Previous research studies explained the lowering in the chlorophyll content at higher Cd doses (Figs. 4a-c) might be due to degradation of chlorophyll or inhibition of its biosynthetic enzymes (Somashekaraiah *et al.*, 1992; Vajpayee *et al.*, 2000). Often, cadmium treatment in rice affects the donor side of the PSII *in vitro* was first evident by Bazzaz *et al.,* 1974. Hence, such damages in the chloroplasts severely hampers with the process of photosynthesis (Bazzaz *et al.*, 1974). There are not much of significant changes of carotinoids in alteration of Cd concentration in all the tested three varieties of rice (Figs. 5a-c).

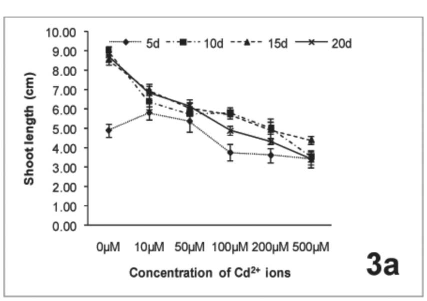


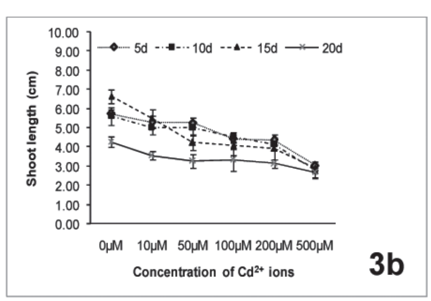


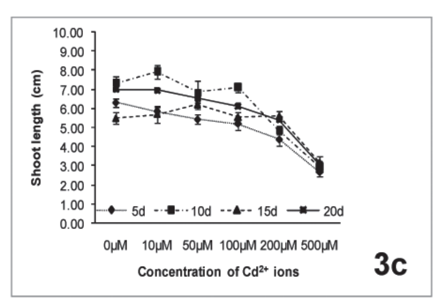


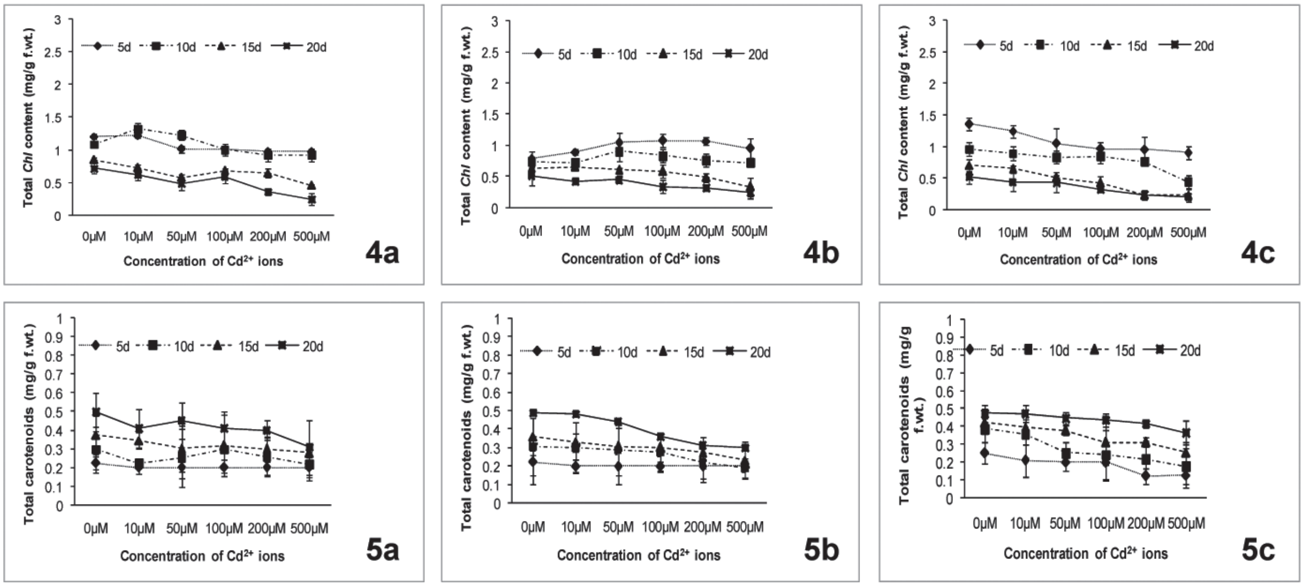












Figs. 1a&b. Differential root and shoot growth responses of var. Hiranmayee (Fig. 2a) and var. Mrunalini (Fig. 2b) at different dosed of Cd treatment. Figs. 2a-c. Effect of different low doses of Cd on root growth of three varieties of rice. Figs. 3a-c. Effect of different low doses of Cd on shoot growth of three varieties of rice. Figs. 4a-c. Effect of different low doses of Cd on *Chl* content of three varieties of rice. Figs. 5a-c. Effect of different low doses of Cd on carotenoid content of three varieties of rice.

4. Conclusion

Increasing heavy metal threat in the field of agriculture makes it essential to investigate the three important paddy crops for their efficacy to cope with the cadmium effects. All the varieties were more or less affected in their plant height and photosynthetic effects. The common phytotoxic effects like chlorosis and root stunting and discolouration was common in all the varieties. Photosynthetic pigment system is also affected, mostly the chlorophyll b. The level of carotenoid content, however, varied according to the chlorophyll content as a means to compensate the pigment loss. It was evident that, var. Hiranmayee was comparatively tolerant while var. Jagannath had the least phytotoxic effect due to the Cd stress.

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