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CHANGES IN MORPHOPHYSIOLOGICAL PARAMETERS AND ANTIOXIDANT SYSTEM UNDER CADMIUM TOXICITY IN MAIZE (*ZEA MAYS* L.)

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ABSTRACT

The present study investigated the effect of cadmium ($CdNO_3$ 100 μM and 200 μM) on morphological, biochemical and antioxidant enzymes in two maize genotypes at early growth stages. It was observed that Cd-exposure decreased plant height, leaf area and dry weight and the decrease was more pronounced at higher doses of cadmium. The maximum decrease in dry weight (62.54 per cent) was recorded in HQPM-1 at 200 μM $CdNO_3$. There was drastic decrease in Chl a and Chl b content. There was upregulation in antioxidant mechanism on Cd-exposure as evidenced by increase in activities of antioxidant enzymes catalase (CAT) and peroxidase (APX). The oxidative damage was observed as there was increase in malondialdehyde content. In maize genotype DKC-7074, CAT played main role, whereas in, HQPM-1, APX played main role in antioxidant mechanism. Among genotypes, HQPM-1 proved to be more susceptible to cadmium stress. The plants became gradually more adaptable and lesser growth reduction was noticed at 100 μM of $CdNO_3$.

INTRODUCTION

Environmental stresses, both biotic and abiotic stresses, are important factors that affect growth and metabolism of plants. Heavy metal pollution has been a cause of major concern in recent days as the modern human activities is aggravating the situation. Heavy metals are metals with a density higher than 5g cm^{-3}. Cadmium is a dangerous heavy metal having density of 8.642 g cm^{-3} at 20° C. Cadmium (Cd) is a highly toxic trace element and has been ranked seventh among the top 20 toxins (Wang *et al*., 2004). In India, cadmium has emerged as a potential pollutant in industrial areas of Bihar, West Bengal, Tamil Nadu and Delhi. Considerable amount of cadmium is released by leather and paint industry in Delhi. Amongst the different metals, cadmium concentration in Yamuna river water was quite noticeable and major portion of it (30–50%) is contained in the most mobile fraction (either exchangeable or carbonate bound) and there fore can easily enter the food chain. Cadmium has toxic effect on animal body and specially in protein metabolism too.(Patnaik *et al*., 2010).

Maize (*Zea mays* L.) is a suitable crop for tropics and subtropical regions of the world. Being a

rich source of nutrition (72% starch, 10% protein, 8.5% fiber, and 4.8% edible oil), maize is a major source of food, sugar,cooking oil, and animal feed all over the globe.(Dowswell *et al*.,1996)Its rapid growth and high biomass accumulation, has made maize as model crop for phytoremediation approach for heavy metal pollution in many studies (Wang *et al*., 2007). Xu *et al*. (2014) reported that Cd stress inhibited maize growth and damaged the chloroplast tissues, but some morphological traits such as dry biomass and leaf symptom were appeared to be insensitive to the Cd stress. The effects of heavy metals on plant depend on the amount of lethal substance taken up from a particular environment(Vitoria *et al*.,2001). The dose and duration, developmental age, species and genotype of the plant on which the stress is imposed all influence the plants responses, its adjustment, environmental fitness to maintain its homeostasis or steady state physiology. It evokes a number of parallel and, or consecutive events at molecular, physiological and morphological levels. There fore, the present study was conducted to study the effect cadmium on different growth parameters, biochemical parameters and antioxidant system in two maize genotypes from germination to 30 days after emergence at 10 days interval. The objective of the study was to study different doses of cadmium on two different maize genotypes at early seedling growth stage.

MATERIALS AND METHODS

The experiment was performed in the Laboratory of the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Disease free, healthy seeds of two maize varieties HQPM-1 and DKC-7074 were procured from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi.

Bold and uniform seeds were selected where as small, discoloured, infected seeds were discarded. HQPM-1 (HKI 193-1 x HKI 163) was popular protein rich variety where as the other variety DKC-7074 was developed by Monsanto, Desi type with attractive colour. Homogenous seeds of two genotypes of maize were surface sterilized in sodium hypochlorite solution (0.1%). Surface-sterilized seeds, four in number were sown in each pots filled with dried soil sterilized by using 70% methanol. Two healthy plants were maintained after emergence in each pot. Morpho-physiological, biochemical and enzymatic activity were recorded at 10, 20 and 30 days after emergence. All the samples for biochemical estimation were taken between 8 A.M. to 9 A.M. in the morning. Leaf samples were collected from top 2nd and 3rd leaf and mixed to make final weight of required quantity of sample for estimation.

Plant height was measured for plants from the base of the plant to the growing tip of main shoot with the help of meter scale, averaged and expressed in centimeter. Leaf area was determined by dry weight method. Two plants were sampled for estimation of leaf area. Leaf area of two leaves were obtained using leaf area meter (Systronics 211) and such leaves along with remaining leaves were dried separately in hot air oven at 80°C for 72 hours. The dry weight of two leaves and rest of the leaves was recorded and the leaf area was calculated by using the following formula: Leaf area = $(a \times w)/b + a$ where, a = leaf area (cm^2) of 2 leaves, b = dry weight (mg) of 2 leaves and w = dry weight (mg) of rest of the leaves. Chlorophyll 'a' and 'b' content in leaves were determined by the method of Arnon, 1949. The Chlorophyll content was estimated by the formulae as given below.

$$\text{Chlorophyll 'a' content (mg/g f. wt.)} = (12.7 \times A663 - 2.69 \times A645) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll 'b' content (mg/g f. wt.)} = (22.9 \times A645 - 4.68 \times A663) \times \frac{V}{1000 \times W}$$

$$\text{Cartenoid' content (mg/g f. wt.)} = 4.49 \times A_{450} - 0.26(2.02 \times A_{645} + 8.02 \times A_{663}) \times \frac{V}{1000 \times W}$$

Ascorbate peroxidase (APX: 1.11.1.11) was assayed as per the protocol of Nakano and Asada, 1981, whereas, Catalase (CAT: 1.11.1.6) activity was assayed as per protocol of Aebi *et al.*, 1983. The enzyme activity was expressed according to the formula given below:

$$\text{Enzyme units} = \frac{\partial A_{230} / \min \times 1000}{43.6 \text{ mL reaction mixture}}$$

The level of lipid peroxidation was estimated as the malondialdehyde (MDA) content, determined according to the method of Heath and Packer, 1968. The amount of MDA–TBA complex (red pigment) was calculated from the extinction coefficient as 155 mM^{-1} cm^{-1}.

MDA content (n mol mL^{-1}) = $\{(A_{532}\text{-}A_{600})/155\} \times 10^3$

Analysis of variance was performed on the data at each stages as described by Panse and Sukhatme (1967). Critical difference (C.D.) values were calculated at 5 percent probability level

RESULTS AND DISCUSSION

In the present study, morphological parameters such as plant height, leaf area and dry weight reduced in both varieties under cadmium toxicity. At all the stages, it was documented that the inhibitory effect of cadmium on these three morphological parameters was less in DKC-7074 than in variety HQPM-1, as well as it was also found that reduction was maximum at higher doses of cadmium. Plant height showed significant differences among different treatments at 10, 20 and 30 days after emergence (DAE) as presented in Table 1. Among treatments, plant height was found maximum in T1 (control), whereas, the least plant height was recorded in treatment T3 (200 μM $CdNO_3$) at all the three growth stages. There was greater decline in plant height in both varieties at higher dose ($CdNO_3$, 200 μM) treatment than T1 (Control). At 30 days after emergence (DAE), there was 22.9 per cent reduction in plant height in DKC-7074 and 30.6 per cent reduction in HQPM-1 over control. Maximum leaf area reduction was recorded at 30 DAE in HQPM-1 (61.9%) as compared to DKC-7074 (53.2%) for $CdNO_3$, 200 μM (Fig.1). The reduction in dry matter accumulation was highest in case of HQPM-1 for $CdNO_3$, 200 μM (62.54%) and for $CdNO_3$, 100 μM (29.86%) on 10 DAE as compared to control (Fig.2). The inhibitory effect of cadmium on growth might be mediated through hindrance in cell growth. Cadmium in cells get associated with middle lamella and increase the cross linking that eventually lead to reduced cellular expansion and growth (Poschenrieder *et al.*, 1983). We also know that turgor pressure has important role in cell expansion. Cadmium also alters the water relation in plant. Cadmium leads to a reduction in Ca^{2+} content (Rivetta *et al.*, 1997) and it is well documented that calcium play pivotal role in cell division, cell wall and plasma membrane maintenance.

The present study revealed that upon exposure to cadmium chlorophyll a and b content decreased significantly ($p < 0.05$) in the leaves of maize genotypes (Fig. 3 and 4). The effect was concentration dependent. The chlorophyll a content decreased by 67.70% and 47.73% at 200 μM and 100 μM $CdNO_3$ respectively, as compared to control in DKC-7074 at 30 DAE (Fig. 3). Our results of decrease in chlorophyll content is similar to the findings of Siedlecka and Krupa (1996) who also found a decrease in chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum*. Cadmium is known to enhance

Table1 : Effect of cadmium stress in plant height.

Variety	Plant height (cm)								
	10 DAE			20DAE			30DAE		
Treatment	DKC-7074	HQPM-1	Mean	DKC-7074	HQPM-1	Mean	DKC-7074	HQPM-1	Mean
Control(T1)	40.33	40.66	40.5	65.5	65.23	65.36	86.9	90	88.41
$CdNO_3$ @ 100 μM(T2)	38.83	35.83	37.33	61.93	60.16	61.05	84.66	84.33	84.5
$CdNO_3$ @ 200 μM(T3)	38.33	36.83	37.58	59	56.83	57.91	67	62.5	62.5
Mean	39.16	37.77		62.14	60.74		80.55	77.88	

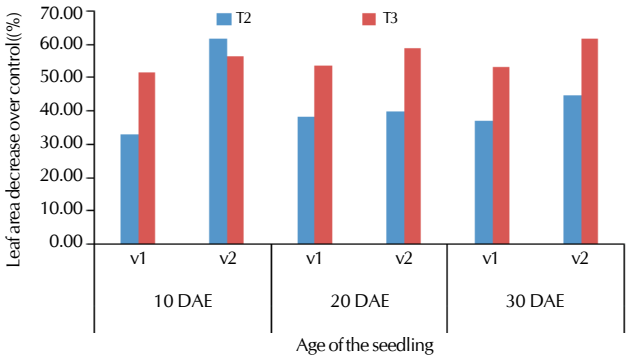

Figure1: Effect of cadmium stress on leaf area

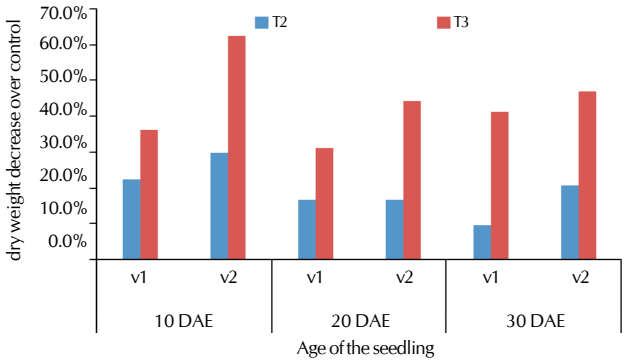

Figure 2: Effect of cadmium stress on dry weight

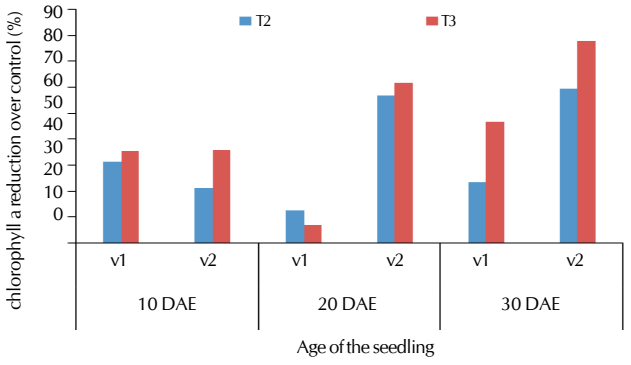

Figure 3: Effect of cadmium stress on chlorophyll a content

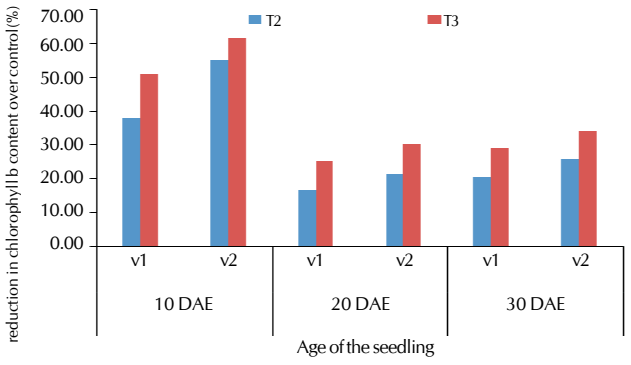

Figure 4: Effect of cadmium stress on chlorophyll b content

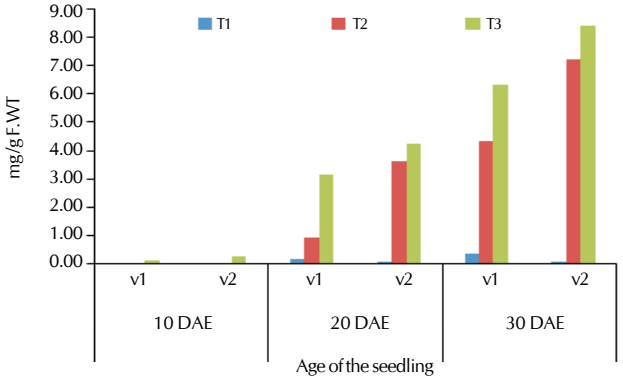

Figure 5: Effect of cadmium on carotenoid content

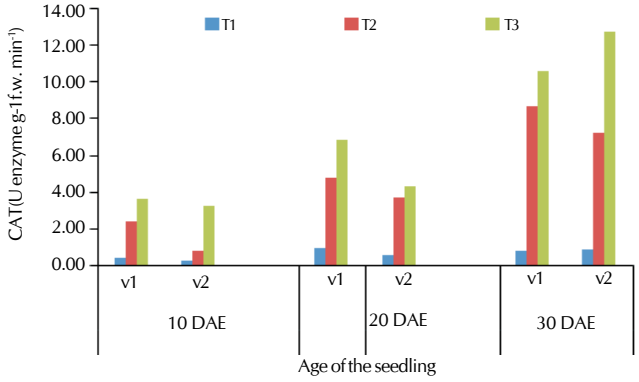

Figure 6: Effect of cadmium doses on Catalase (CAT) enzume activity

the levels of enzyme chlorophyllase that causes the degradation of chlorophyll .Our result is also in accordance with the finding of Hemlata *et al.*, 2010 who find similar result in finger millet seedlings.The decline in chlorophyll content in plants exposed to cadmium stress is believed to be due to: (a) inhibition of important enzymes, such as ä-aminolevulinic acid dehydratase (ALAdehydratase) and proto chlorophyllide reductase (Van Assche and Clijsters1990) associated with chlorophyll biosynthesis; or due to impairment in the supply of Mg^{2+} and Fe^{2+} required for the synthesis of chlorophylls; (c) Zn^{2+} deficiency resulting in inhibition of enzymes, such as carbonic anhydrase (Van Assche and Clijsters, 1990). On the other hand, carotenoid content increased with the increasing Cd concentration (Fig. 5). The highest carotenoid content was measured at $CdNO_3$ 200 μM concentration at 30 DAE. Our findings are in contrast to results reported by Yasemin Ekmekci *et al.*, 2006 where it decreased with increasing Cd concentration.

In our study, we observed that CAT and APX content increased with the doses of cadmium in both genotypes (Fig. 6 and 7). The increase in CAT and APX activity was pronounced even at lower Cd concentration of 100 μM as compared to control. Dipankar *et al.* (2007) and Shah *et al.* (2001) also found similar type of results in ground nut and rice seedlings respectively and supports our experimental observations. Low Cd concentrations and short treatment periods generally produce an increase in antioxidative enzymes (Smeets *et al.*, 2005). Cd regulates CAT at both transcriptional and posttranscriptional

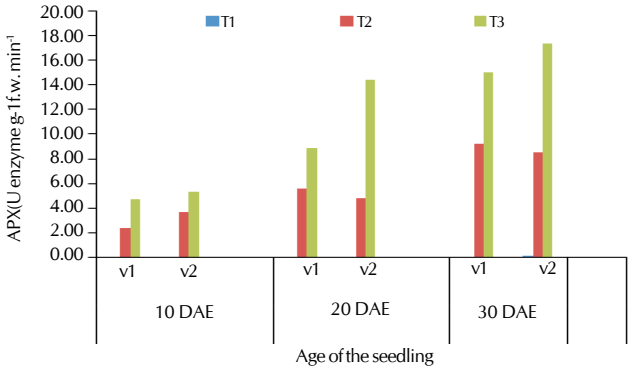

Figure 7: Effect of cadmium doses on Ascorbate peroxidase (APX) enzyme activity

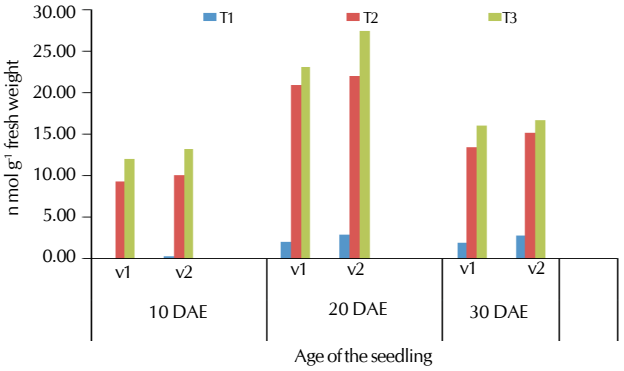

Figure 8: Effect of cadmium doses on malondealdehyde (MDA) content

level (Rodríguez-Serrano *et al.,* 2006). In our study more catalase activity was recorded in DKC-7074 and proved more adaptable with oxidative damage caused by cadmium. In HQPM-1 we found more amount of APX production than DKC-7074 although in both varieties sharp increase in APX production was recorded at treatment $Cd(NO_3)_2$ 200 μM.

MDA level, altered due to Cd-induced oxidative stress, was assessed as an index of Cd toxicity. In our experiment we saw that HQPM-1 produced more MDA than DKC-7074 for both the treatment in all three stages (Fig. 8). The increase in MDA content was sharp at 100 μM Cd concentration itself (approx. 10 times) as compared to control which increased marginally at 200 μM concentration. The presence of high amount of MDA in HQPM-1 could be explained with the fact that low levels of antioxidant enzyme activities may result in the enhancement of free radical-mediated lipid peroxidation (Foyer, 1987). So it may be concluded that may be the genotype HQPM-1 is not capable of producing enough antioxidant enzymes that can prevent oxidative damage and lipid peroxidation of membrane caused by cadmium.

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