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SODIUM CHLORIDE AND CADMIUM INDUCED OXIDATIVE STRESS AND ANTIOXIDANT RESPONSE IN *CALAMUS TENUIS* LEAVES

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SUMMARY

The effects of NaCl (0-300 mM), $CdCl_2$ (0-10 mM) and NaCl (300 mM) + $CdCl_2$ (10 mM) induced oxidative stress in *Calamus tenuis* Roxb. leaves were studied. Alterations in the activated oxygen metabolism was detected as evidenced by the increased peroxide content and lipid peroxidation due to the accumulation of thiobarbituric acid reactive substances (TBARS) with the increasing NaCl and Cd concentrations. Superoxide dismutase (SOD) and peroxidase (POX) decreased, while catalase (CAT) and glutathione reductase (GR) activities increased during NaCl treatments. Cd treatment decreased SOD and CAT activities, while POX and GR activities increased. NaCl + Cd treatment decreased all enzyme activities except GR. Ascorbate and glutathione content increased in response to NaCl and Cd treatments, whereas, NaCl + Cd treatment decreased ascorbate and increased glutathione content. The cadmium uptake increased up to 10 times in 10 mM treatment as compared to lower concentrations. Enhanced levels in some of the antioxidants observed in the investigation suggest that a better antioxidant defense is required for higher NaCl and cadmium tolerance.

***Key words*: Antioxidant enzymes, *Calamus tenuis*, cadmium, sodium chloride.**

INTRODUCTION

Increased salinity and cadmium toxicity lead to significant decrease in crop productivity. Salinity limits plant growth and productivity affecting water deficit, ionic imbalance, osmotic stress and the secondary induced oxidative stress by the production of reactive oxygen species (ROS) (Khan *et al.* 2002, Panda and Khan 2003, Flowers 2004, Demiral and Turkan 2005, Mandhania *et al*. 2006). Cadmium is one of the most dangerous heavy metals due to its high mobility and the small concentration at which its effects on plants begin to show. Cadmium preferably accumulated in the roots and decreases the water uptake in roots, however, Cd accumulates in the leaves. Cadmium produces alterations in the functionality of membranes by inducing changes in lipid composition and the toxicity of Cd has been related with the increase in lipid peroxidation and alterations in antioxidant systems (Sandalio *et al*. 2001, Astolfi *et al*. 2004, Chaoui *et al*. 2004, Srivastava *et al*. 2004).

Any imbalance in the cellular redox homeostasis can be called oxidative stress which results in the production of reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$), alkoxyl radical ($RO^{\cdot}$) and singlet oxygen ($^{\cdot}O_2$) formation via enhanced leakage of electron to oxygen. ROS attack proteins, lipids and nucleic acids. Plants have developed a complex antioxidative defence systems to alleviate the damage caused by ROS and the degree of damage depends on the balance between the formation of ROS and its removal by the antioxidative scavenging

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systems that defend against them. The antioxidative system includes carotenoids, ascorbate, glutathione, α-tocopherols and enzymes such as superoxide dismutase, catalase, glutathione reductase, peroxidases and enzymes involved in ascorbate glutathione (ASC-GSH) cycle, ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase and glutathione reductase (Sandalio *et al*. 2001, Khan *et al*. 2002, Bor *et al*. 2003, Panda and Khan 2003, Astolfi *et al*. 2004, Chaoui *et al*. 2004, Srivastava *et al*. 2004, Demiral and Turkan 2005, Mandhania *et al*. 2006).

Calamus tenuis (rattan) is a tropical climbing palm that grows under various habitats and has multiple economic uses. The effect of NaCl and Cd induced oxidative stress and antioxidant metabolisms in rattan are relatively less known. The present investigation was carried out to evaluate NaCl, Cd and NaCl + Cd induced oxidative stress, antioxidant responses and tolerance to NaCl and Cd in rattan leaves.

MATERIALS AND METHODS

Rattan (*Calamus tenuis* Roxb.) seeds were sown and germinated in a plastic tray containing sand moistened with tap water for four weeks. The germinated seeds one in each was transferred to plastic pots containing sand and grown with half strength nutrient Hoagland's solution in green house for 9 (nine) months. The environmental conditions in the green house containing the germinated plants were 28 ^{0}C / 24 ^{0}C (day / night) temperature, 80 % relative humidity and 7000 lux of light intensity with 18h photoperiod. The 9 months old plants were treated with NaCl (0, 100, 200 and 300 mM), cadmium chloride (0.1, 1 and 10 mM). To evaluate the effects of highest NaCl and Cd together, NaCl (300 mM + Cd (10 mM) were used. The treatments were allowed in half strength Hoagland's nutrient solution in soil for 5 (five) days. Seedling grown in soil with half strength Hoagland's nutrient solution only was used as control. The experimental design was repeated twice with three replicates (individual glasses). On 6th day, the young leaves were used for various biochemical estimations. Leaf (0.5 g) tissue was homogenized in 5 % trichloroacetic acid (TCA) and the homogenate was used for the determination of hydrogen peroxide (H_2O_2) levels by the method of Sagisaka (1976). The level of lipid peroxidation was determined by the amount of malondialdehyde (MDA) chiefly thiobarbituric reactive substances (TBARS) accumulation as described (Heath and Packer, 1968). For the extraction and estimation of ascorbate, the method of Oser (1979) was used. Glutathione was extracted and estimated as per the method of Griffith (1980). Leaf tissue (0.2 g) was homogenized at 4 ^{0}C in 5 ml chilled extraction buffer (0.1 M phosphate buffer, pH 6.8) with mortar and pestle. The homogenate was then centrifuged at 15,000 g for 20 min and the homogenate was used as the crude extract for the assay of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and glutathione reductase (GR) as per the methods of Giannopolitis and Reis (1977), Chance and Maehly (1955) and Smith *et al*. (1988) respectively. Values presented in the experiment are means of two independent experiments with three replicates each ± standard errors of mean (±SEM).

RESULTS AND DISCUSSION

The effects of increasing concentrations of NaCl (0-300 mM), Cd (0-10 mM) and NaCl (300 mM) + Cd (10 mM) together for 5 d in the leaves of rattan showed increased peroxide content (Fig. 1). As an indicator of lipid peroxidation, the content of thiobarbituric acid reacting substances (TBARS) was measured. Increasing concentrations of NaCl, Cd and NaCl + Cd together caused an enhancement of TBARS (Fig. 1). Romero-Puertas *et al*. (1999) demonstrated that Cd produces about twice the amount of H_2O_2 levels in peroxisomes purified from pea plants exposed to 50 mM Cd. Piqueras *et al*. (1999) have detected a fast generation of H_2O_2 in tobacco cell cultures in response to 5 mM Cd. NaCl, Cd and NaCl + Cd together showed enhanced TBA-reacting substances (Fig. 1), an index of lipid peroxidation and is produced when unsaturated fatty acids in the membrane undergo oxidation by the accumulation of free radicals and therefore oxidative stress. Similar increases of TBARS in salt treatments have been observed in various plants (Khan *et al*. 2002, Khan and Panda 2002, Bor *et al*. 2003, Meloni 2003, Panda and Khan 2003) and Cd exposure has been observed in various plants (Cho and Park 1999, Sandalio *et al*. 2001). The peroxidation of cell membranes severely affects its functionality and integrity and can produce irreversible damage to cell function and

Fig. 1. Changes in peroxide levels and thiobarbituric acid reactive substances (TBARS) content subjected to NaCl (0, 100, 200, 300 mM), Cd (0, 0.1, 1, 10 mM) and NaCl (300 mM) + Cd (10 mM) treatments in *Calamus tenuis* leaves. Data represent mean ± SEM.

Fig. 2. Changes in ascorbate and glutathione content subjected to NaCl (0, 100, 200, 300 mM), Cd (0, 0.1, 1, 10 mM) and NaCl (300 mM) + Cd (10 mM) treatments in *Calamus tenuis* leaves. Data represent mean ± SEM.

can be initiated by ROS species such as $O_2^{\cdot}$, $^{\cdot}OH$, H_2O_2 or by the action of lipoxygenase. Cd is not a redox metal, like Cu and Fe, and therefore cannot catalyze Fenton-type reaction yielding ROS. However, Cd can induce oxidative stress indirectly by producing disturbances in chloroplasts. Salt stress produce ion leakage indicating injury to membranes integrity, which could be affected by ROS formed during leaf photosynthesis or respiration, enhancing lipid peroxidation of the membranes (Cho and Park 1999).

The non-enzymic antioxidant ascorbate and glutathione react directly with ROS in photosynthetic tissues, recycles α-tocopherol and protect enzymes with prosthetic metals ions and is utilized as a substrate for ascorbate peroxidase which catalyzes H_2O_2 detoxification. Though NaCl (300 mM) + Cd (10 mM) together showed decreased ascorbate and glutathione content, however, increased significantly with the increasing concentrations of NaCl (0-300 mM) and Cd (0-10 mM) treated leaves (Fig. 2) allowing better antioxidant protection as reported for other plants (Shalata *et al*. 2001, Khan and Panda 2002, Khan *et al*. 2002). Glutathione as non-enzymic antioxidant to ROS plays a protective role by increasing stress tolerance in particular that of salinity and oxidative stress and seems to be an important signal molecule by acting as a direct link between environmental stress and key adaptive responses. Increased glutathione redox state may serve as signal affecting the expression of defensive genes. Changes in processes that regulate GSH concentrations and / or redox status are considered to be one of the important adaptive mechanisms of plant exposed to stress conditions. The increased level of GSH during Cd stress may possibly be due to induced transcription of the genes for GSH biosynthesis such as α-glutamylcysteine synthetase, glutathione synthetase and glutathione reductase (Xiang and Oliver 1998). Cadmium stressed plants have to restore the GSH used to form PCs. According to Sharma *et al*. (2004) the incorporation of GSH into PCs could be compensated by an augmented sulfur uptake to support GSH biosynthesis.

Superoxide dismutase (SOD) catalyzes the dismutation of O_2^{-} to H_2O_2 and O_2. SOD is key enzyme

in protecting cells from oxidative stress. There is a significant decrease in SOD activity in all the treatments (Fig. 3), thereby lowering the dismutation of O_2^- and unabling the plant to resist the potential oxidative damage caused by the NaCl and Cd exposure. NaCl and Cd induced reduction in SOD activity has been observed in various plants (Sandalio *et al*. 2001, Khan *et al*. 2002, Panda and Khan 2003) while an enhancement of SOD activity in response to moderate Cd concentrations have been reported in *Lemna* plants (Srivastava and Tel-Or 1991). Pereira *et al*. (2002) studying with *Crotalaria juncea* in response to Cd showed no change in SOD activity. Reduction in SOD activity at higher NaCl and Cd treatments may be attributed to an inactivation of enzyme by H_2O_2, which is produced in different cellular compartments and also from a number of non-enzymatic and enzymatic processes in cells (Sandalio *et al*. 2001, Schutzendubel *et al*. 2002). Peroxidases are involved not only in scavenging of H_2O_2 produced in chloroplasts but also in growth and developmental processes. POX activity significantly decreased in all the treatments, whereas, lower Cd treatments increased POX activity (Fig. 3). Increased and decreased POX activity has been reported in various plants (Sandalio *et al*. 2001, Khan and Panda 2002, Khan *et al*. 2002, Bor *et al*. 2003, Demiral and Turkan 2005). POX activity showed better response to Cd as compared to NaCl, thus seemed to play a lead role in Cd-induced oxidative damage as compared to NaCl induced oxidative damage. POX is thought to be a stress marker enzyme and its higher induction may indicate stress exerted by heavy metal, which can be correlated with amount of the accumulated metal (Shigeoka *et al*. 2002, Chaoui *et al*. 2004, Srivastava *et al*. 2004).

Catalase (CAT) removes H_2O_2 by catalyzing H_2O_2 to H_2O. An increased CAT activity was observed in both NaCl and Cd treated leaves, however, higher Cd concentrations and NaCl + Cd decreased CAT activity significantly (Fig. 4). Similar results were observed by various authors in several plants (Sandalio *et al*. 2001, Pereira *et al*. 2002, Vaidyanathan *et al*. 2003, Mishra *et al*. 2006). Under higher Cd and NaCl + Cd treatments, CAT diminished, the plants are not fully competent to remove the H_2O_2 which would accumulate to toxic levels. Glutathione reductase (GR) a key enzyme of the

Figure 3. Changes in superoxide dismutase (SOD) and peroxidase (POX) activities subjected to NaCl (0, 100, 200, 300 mM), Cd (0, 0.1, 1, 10 mM) and NaCl (300 mM) + Cd (10 mM) treatments in *Calamus tenuis* leaves. Data represent mean ± SEM.

Figure 4. Changes in catalase (CAT) and glutathione reductase (GR) activities subjected to NaCl (0, 100, 200, 300 mM), Cd (0, 0.1, 1, 10 mM) and NaCl (300 mM) + Cd (10 mM) treatments in *Calamus tenuis* leaves. Data represent mean ± SEM.

ascorbate-glutathione cycle increased in NaCl and Cd treated leaves, however, higher NaCl (300 mM), Cd (10 mM) and NaCl + Cd treatments showed significant decrease in GR activity (Fig. 4). Increased and decreased GR activity has been observed in response to NaCl and Cd stress in various plants (Mittova *et al*. 2000, Vitoria *et al*. 2001, Pereira *et al*. 2002, Bor *et al*. 2003). Schickler and Capsi (1999) reported increased GR activity at low levels of Cd in *Alyssum argenteum*, considered as a metal-hyperaccumulator plant while the GR activity was reduced at higher Cd concentrations. The increased activity of GR could be explained by transcriptional or translational modification to keep an adequate GR level to protect against Cd stress (Romero-Puertas *et al*. 2002).

The cadmium uptake increased with the increasing Cd (01, 1 and 10 mM) concentrations and the uptake was 10 fold high in 10 mM Cd treated leaves (Fig. 5). Cho and Park (1999) observed similar results in tomato. The combined effect of NaCl (300 mM) + Cd (10 mM) treatments showed decreased uptake compared to 10 mM Cd treatments (Fig. 5). In leaves, Cd accumulation may be driven by active transpiration. It has been suggested that the stem behaves as a cation exchange column resulting in a chromatographic distribution of metals towards the leaves and top of the plants, amount of Cd absorbed by the plants could be elevated by inducing higher transpiration rates (Vitoria *et al*. 2001, Fargasova *et al*. 2001).

Figure 5. Cadmium uptake subjected to NaCl (0, 100, 200, 300 mM), Cd (0, 0.1, 1, 10 mM) and NaCl (300 mM) + Cd (10 mM) treatments in *Calamus tenuis* leaves. Data represent mean ± SEM.

In conclusion, the C*alamus tenuis* leaves treated with NaCl, Cd and NaCl + Cd induced a concentration-dependent oxidative stress characterized by an increased uptake of Cd, accumulation of peroxides and lipid peroxidation. NaCl + Cd decreased the enzyme activities, ascorbate and glutathione content. Though SOD and POX (NaCl treated leaves) activities decreased, an increased CAT, POX, (Cd treated leaves) GR activities, ascorbate and glutathione content (NaCl and Cd treated leaves) displaying better antioxidant defense. Enhanced levels in some of the antioxidants observed in the investigation suggest that a better antioxidant defense is required for higher NaCl and cadmium tolerance.

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