Changes in growth and superoxide dismutase activity in *Hydrilla verticillata* L. under abiotic stress

Sanjib Kumar Panda[1*] and Mohammad Hussain Khan[2]

*[1]Research Institute for Bioresouces, Okayama University, Kurashiki, 710-0046 Japan, [2]Plant Biochemistry Laboratory, School of Life Sciences, Assam (Central) University, Silchar –788011, Assam, India; *Corresponding author: drskp@rib.okayama-u.ac.jp*
Received: 21/05/2004, Accepted: 02/07/2004

A study was made of the role of various heavy metals (chromium, zinc, copper and cadmium) together with the effects of NaCl-salinity, PEG-mediated water stress and temperature on growth and superoxide dismutase (SOD) activity in hydrilla (*Hydrilla verticillata* L.). Almost all metals studied reduced growth but presented a modulatory effect at lower concentrations. NaCl-salinity, water stress and temperature treatments also reduced growth. SOD activity was inhibited by both chromium and zinc but increased by copper and cadmium. NaCl-salinity increased SOD activity while water stress decreased it. SOD showed thermostability with a higher activity being recorded at 45°C.
Key words: growth, heavy metals, NaCl, PEG, superoxide dismutase, temperature.

Alterações no crescimento e na atividade da dismutase do superóxido em *Hydrilla verticillata* L. sob estresse abiótico: Estudaram-se os efeitos de alguns metais pesados (cromo, zinco, cobre e cádmio), da temperatura, da salinidade induzida por NaCl e do estresse hídrico induzido por PEG sobre o crescimento e a atividade da dismutase do superóxido (SOD) em *Hydrilla verticillata* L. Quase todos esses metais reduziram o crescimento, mas tiveram efeito modulador em baixas concentrações. Os tratamentos de salinidade induzida por NaCl, estresse hídrico e temperatura, também o reduziram. A atividade do SOD foi inibida por cromo e zinco, mas aumentada por cobre e cádmio. O estresse salino aumentou a atividade do SOD, enquanto o hídrico o diminuiu. O SOD mostrou-se termoestável, sendo a maior atividade atingida a 45°C.
Palavras-chave: crescimento, dismutase do superóxido, metais pesados, NaCl, PEG, temperatura.

Plants in the environment are exposed to a range of abiotic stresses like osmotic, salinity, temperature and heavy metal toxicity, which affect their growth and other physiological processes (Levitt, 1980).

Abiotic stresses are known to act as a catalyst in producing free radical reactions resulting in oxidative stress in various plants where reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydroxyl radical (·OH), hydrogen peroxide (H_2O_2) and alkoxyl radical (RO·) are produced (Scandalios, 1993; Zhang and Kirkham, 1994; Hernandez et al., 1994; Gallego et al., 1996; Weckx and Clijsters, 1997; Loggini et al., 1999; Panda and Patra, 2000; Bakardjieva et al., 2000; Hernandez et al., 2000. The toxic superoxide radical has a half life of less than one second and is usually rapidly dismutated by superoxide dismutase (SOD) to H_2O_2, a product which is relatively stable and can be detoxified by catalase (CAT) and peroxidases (Grant and Loake, 2000). Increased SOD activity is known to confer oxidative stress tolerance (Bowler et al., 1992; Slooten et al., 1995). If the production rate of the superoxide radical under abiotic stress exceeds SOD activity, then oxidative damage results (Casano et al., 1997). Therefore, the present investigation was carried out in order to understand the influence of various abiotic stresses on growth and SOD activity of the aquatic macrophyte plant hydrilla (*Hydrilla verticillata* L.).

Hydrilla was collected from a nearby uncontaminated pond and grown in laboratory conditions for 4 days. Freshly growing hydrilla plants (0.2 g) were placed in Petri dishes containing different concentrations (0, 10, 100 and 1,000 µmol.L^{-1}) of heavy metal solutions of $CdCl_2$, $CuCl_2$, $ZnSO_4$ and K_2CrO_7. Water stress was imposed by the application of PEG-6000 (0, 10, 20 and 30%). Salinity stress was obtained by NaCl (0, 1, 2 and 3%) application and for temperature treatments the plants were kept at 35°C, 45°C, 55°C while 25°C was used as control. Petri dishes were kept for 24 h under continuous light at 25 ± 2°C. Light was provided by white fluorescent tubes (Philips 36 W TLD) emitting a photon flux density of 52 µmol.m^{-2}.s^{-1}. After 24 h of treatment with the various abiotic stresses, plants were harvested for the determination of growth and SOD activity. Fresh and dry weight were recorded after washing the plants with distilled water and blotting with absorbent paper. For dry weight the plants were left in an oven at 70°C for 48 h before weighing. Proteins were extracted from fresh tissue (0.2 g) with phosphate buffer (pH 6.8, 4°C), centrifuged (17,000 g_n for 15 min at 4°C) and the supernatant used to assay SOD according to Giannopolitis and Ries (1977).

The effect of heavy metals, salinity, water and temperature stresses on fresh and dry weights of hydrilla plants are shown in figure 1. In general all treatments reduced plant growth as concentrations or temperature were increased. There was a decrease in the plant fresh weight (figure 1A) with the increase in concentration of metals though lower concentrations of some metals proved to be beneficial. Compared with fresh weight, the decrease in dry weights were more pronounced as metal concentrations increased (figure 1B). The hydrilla plants seemed to be more sensitive to osmotic stress obtained with PEG (figure 1D) than to salt and temperature stresses (figures 1C and 1E). Osmotic stressed plants also induced a more pronounced decrease of the dry weight. Therefore it would appear that the aquatic plant hydrilla is sensitive to various abiotic stresses with a resulting reduction in growth. Such response is seen for other macrophytes under similar conditions and this may be stress imposed and related to species specific inhibition of hormonal metabolism and plant water relations (Rout and Shaw, 2001a).

Figure 2A shows the changes in the SOD activity under different concentrations of different metal solutions. There was a uniform increase in SOD activity in Cd- and Cu-treated plants whereas a uniform decrease in Zn- and Cr-treated plants was observed with the increase in metal concentration as compared to control. A decrease in SOD activity in hydrilla under Cr and Zn treatment might indicate production of ROS that inactivated SOD protein as reported elsewhere for other plants (Gallego et al., 1996; Casano et al., 1997; Panda and Patra, 2000). However, both Cu and Cd treatments showed

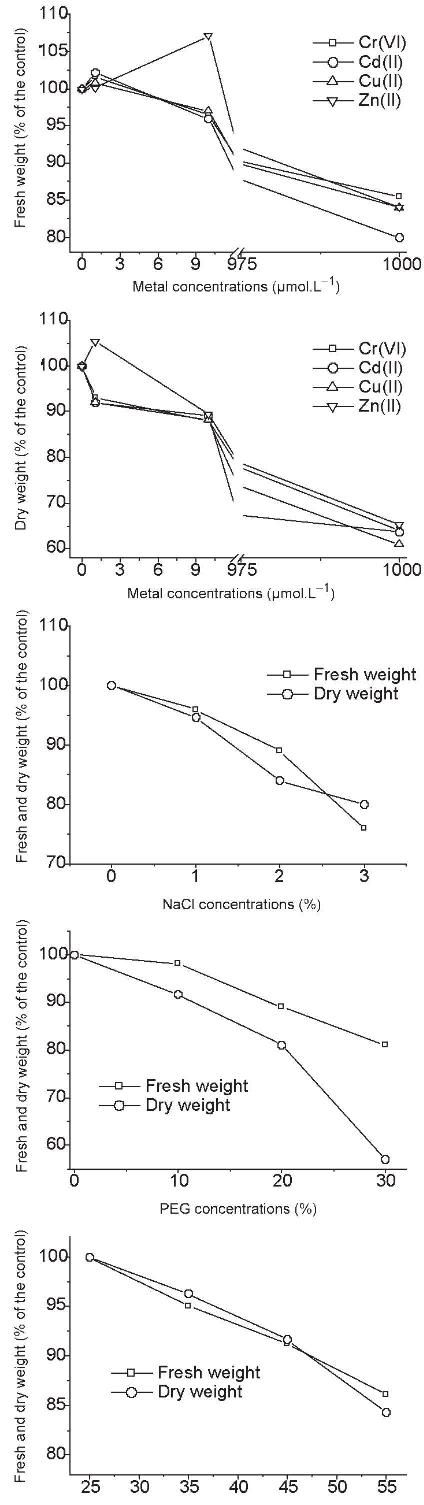

Figure 1. Changes in the fresh weight and dry weight in *Hydrilla verticillata* under different heavy metals (A,B), NaCl (C), PEG (D) and temperature (E) treatments. Data are means of three separate experiments, with 5 replicates each and given as percentage (%) of the control treatments. 100% control values for fresh and dry weight correspond to 0.2 g and 0.018 g, respectively.

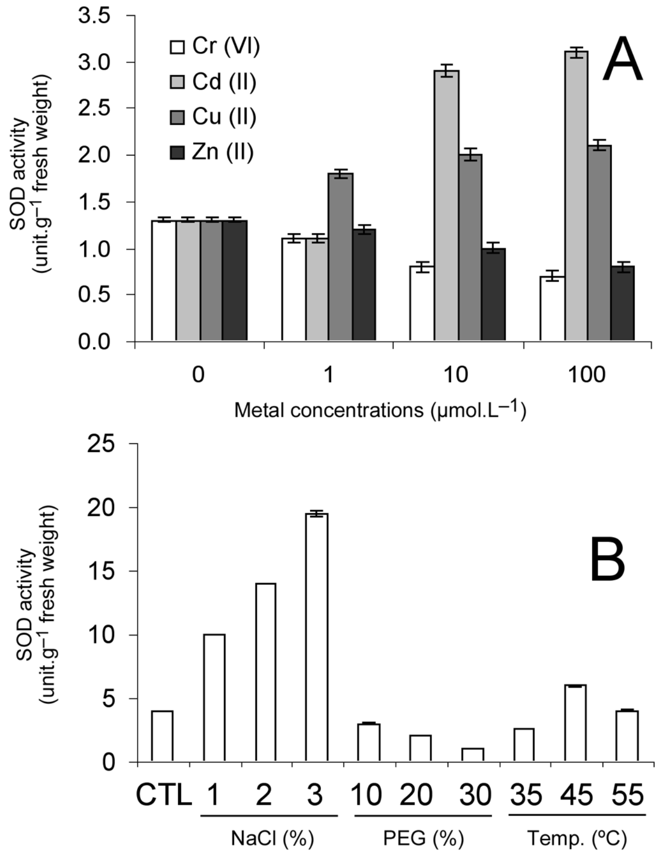

Figure 2. Changes in the superoxide dismutase (SOD) activity in *Hydrilla verticillata* under different heavy metals (A) and NaCl, PEG and temperature (B) treatments, respectively. Data are means of three separate experiments, with 5 replicates each. CTL = control.

an increased SOD activity. Increased SOD activity in oat leaves by Cu have been reported and excess of Cu induces cytosolic Cu/Zn SOD in soybean root (Chongpraditnum et al., 1992; Luna et al., 1994). That Cu induced an increase in SOD activity might be explained by the increase in level of superoxide radicals (Chongpradtinum et al., 1992; Luna et al., 1994). Although Gallego et al. (1996) reported a decrease in SOD activity under Cd treatment, such treatment led to increased SOD activity in pea leaves (Dixit et al., 2001).

Figure 2B depicts the changes of SOD activity under the different concentrations of NaCl, PEG and at different temperature levels. A uniform decrease in SOD activity was recorded with the increase in the PEG concentrations at 10, 20 and 30 %. There was an increase in SOD activity with increasing NaCl concentration, while temperature shocked plants showed a similar trend except for the fall at 55⁰C. The increase in SOD activity under NaCl-salinity stress in hydrilla suggests better oxidative stress tolerance. However, increased water stress caused a uniform decrease in SOD activity, as reported elsewhere for other plants (Smirnoff 1993; Zhang and Kirkham 1994; Navarri-Izzo et al., 1997; Rout and Shaw, 2001b). SOD showed significant thermostability with an increase in SOD activity up to 45⁰C. However, beyond this temperature activity fell as reported for other higher terrestrial plants and lower plants, possibly in view of the heat denaturation of the enzyme being dependent on its sub-unit structure (Kanematsu and Asada 1989; Barkasdjieva et al., 2000).

REFERENCES

Barkasdjieva NT, Chrostov KN, Christina KN (2000) Effect of calcium and zinc on the acivity and thermostability of superoxide dismuatse. Biol. Plant. 43:73-78.

Bowler C, Montagu MV, Inze D (1992) Superoxide dismutase and stress tolernace. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:83-116.

Casano L, Gomez L, Lascano C, Trippi V (1997) Inactivation and degradation of Cu/ZnSOD by active oxygen species in wheat chloroplasts exposed to photooxidative stress. Plant Cell Physiol. 38:433-440.

Chongpraditnum P, Mori S, Chino M (1992) Excess copper induce a cytosolic Cu, Zn superoxide dismutase in soybean root. Plant Cell Physiol. 33:239-244.

Dixit V, Pandey V, Shyam R (2001) Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. *Azad*). J. Exp. Bot. 52:1101-1109.

Gallego SM, Benavides MP, Tomaro ML (1996) Effect of heavy metal ion excess on sunflower leaves : evidence for involvement of oxidative stress. Plant Sci. 121:151-159.

Giannopolitis CN, Reis SK (1997) Superoxide dismutase I. Occurrence in higher plants. Plant Physiol. 59:309-314.

Grant JJ, Loake GJ (2000) Role of active oxygen intermediates and cognate redox signaling in disease resistance. Plant Physiol. 124:21-29.

Hernandez JA, del Rio LA Sevilla F (1994) Salt stress induced changes in superoxide dismutase isozymes in leaves and mesophyll protoplasts from *Vigna radiata* (L.) Walp. New Phytol. 126: 37-44.

Hernandez JA, Jimenez J, Mullineaux P Sevilla F (2000) Tolerance of pea (*Pisum sativum* L.) to long term stress is associated with induction of antioxidant defences. Plant Cell Environ. 23: 583-862.

Kanematsu S, Asada K (1989) Cu/Zn superoxide dismutase in rice : occurrence of an active, monomeric enzyme and non-photosynthetic tissue. Plant Cell Physiol. 30:381-391.

Levitt J (1980) Plant Responses to Environmental Stress. Vol.I. Academic Press, London.

Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F (1999) Antioxidant defense system, pigment composition and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol. 119:1091-1099.

Luna CM, Claudio A, Gonzalez CA, Trippi VS (1994) Oxidative damage caused by an excess of copper in oat leaves. Plant Cell Physiol. 35:11-15.

Navari-Izzo F, Quartracci M, Sgherri C (1997) Dessication tolerance in higher plants related to free radical defences. Phyton 37:203-214.

Panda SK, Patra HK (2000) Does chromium (III) produce oxidative stress in excised wheat leaves ? J. Plant Biol. 27:105-110.

Rout NP, Shaw BP (2000a) Salt tolerance in aquatic macrophytes : possible involvement of the antioxidant enzymes. Plant Sci. 160:415-423.

Rout NP, Shaw BP (2000b) Salt tolernace in aquatic macrophytes: ionic relation and interaction. Biol. Plant. 44:95-99.

Scandalios JG (1993) Oxygen stress and superoxide dismutase. Plant Physiol. 101:7-12.

Slooten L, Capiau K, Van Camp W, Van Montagu M, Sybesma C, Inze D (1995) Factors affecting the enhancement of oxidative stress tolernace in transgenic tobacco over-expressing manganese superoxide dismutase in the chloroplasts. Plant Physiol. 107:373-380.

Smirnoff N (1993) The role of active oxygen in response to water deficit and dessication. New Phytol.125:27-58.

Weckx JEJ, Ciljsters HM (1997) Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris* L. Plant Physiol.Biochem. 35:405-410.

Zhang J, Kirkham MB (1994) Drought-stress induced changes in activities of superoxide dismutase, catalase and peroxidases in wheat leaves. Plant Cell Physiol. 35:785-791.

Zhang J, Kirkham MB (1996) Enzymatic responses of the ascorbate-glutathione cycle to drought in sorghum and sunflower plants. Plant Sci. 113:139-147