



Hydrogen Peroxide and Peroxidase Activity as Potential Indicators on Adaptability of Plants to Salinity Stress Condition

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Abstract The purpose of this study is to investigate whether hydrogen peroxide content as well as peroxidase enzyme activity in the leaves of the cultivars can be used as indicators of the adaptability of plants to salinity stress conditions. For this purpose, leaves of 6 cultivated plant species: *Cassia siamea*, *Acacia auriculiformis*, *Acacia magium*, *Pithecellobium dulce*, *Combretum quadrangulare* and *Albizia lebbek* were collected from previously high salinity areas where the salinity of soil have been improved by plant cultivation, and the relationships between soil characteristics, plant species and the content of hydrogen peroxide and peroxidase enzyme activity were examined. The results show that *C. quadrangulare* has low hydrogen peroxide content and high peroxidase enzyme activity compared to other plants at the same electrical conductivity (0.16 dS m^{-1}), suggesting that this plant has less stress than the others. In other words, *C. quadrangulare* can adapt to high salinity stress conditions better than the others. *A. magium* and *A. auriculiformis* are the second best. Based on the present results, *C. quadrangulare* is the best promising species for growing on high salinity areas.

Keywords salt affected area, hydrogen peroxide, peroxidase enzyme activity

INTRODUCTION

Soil salinity is one of the most significant abiotic stress factors that decrease productivity. High salt concentration in soil or in water affects plant growth, nutrient uptake and metabolism through the decrease in the amount of water available for plants, through ion imbalance or disturbances in ion homeostasis by disturbance of essential intracellular ion concentrations, and through ion toxicity due to excessive Na^+ or Cl^- uptake (Greenway and Munns, 1980; Gossett et al., 1994; Zhu, 2001; Parida et al., 2004). An excess of exchangeable Na ion is harmful to plants, principally because it induces undesirable physical and chemical conditions of the soil. In addition, Na influences soil structure, resulting in a decrease of water infiltration and gas exchangeability. Salinity affects the growth of plants by decreasing the availability of water to the roots due to the osmotic effect of external salt and by toxic effects of excessive salt accumulation within the plant. Drastic changes in

ion and water homeostasis lead to molecular damage, growth arrest and even death (Munns, 1993; Munns et al., 1995).

There is now conclusive evidence that production of reactive oxygen species (ROS) is enhanced in plants in response to different environmental stress conditions such as salinity, drought, water logging, temperature extremes, high light intensity, herbicide treatment and mineral nutrient deficiency. The most stable ROS is hydrogen peroxide (H_2O_2), which is highly reactive and can cause serious oxidative damages to membrane lipids leading to loss of membrane integrity and increase in electrolyte leakage and cell death. ROS were considered to be toxic by-products of aerobic metabolism (Wise and Naylor, 1987; Mittova et al., 2000; Zhu, 2001; Mittova et al., 2002; Slesak et al., 2008).

Salt tolerant plants maintain their growth and have many mechanisms to maintain ionic homeostasis, osmotic homeostasis and detoxification pathways (Dubey, 1994; Zhu, 2001; Parida and Das, 2004). It is known that plants containing high concentration of antioxidants show considerable resistance to the oxidative damage caused by free radicals (Ashraf and Harris, 2004; Parida and Das, 2004; Slesak et al., 2008).

Several authors have discussed the effect of salt stress on the amount of H_2O_2 . One mechanism that underlies the tolerance of plants to salt stresses is the ability of plants to detoxify ROS and scavenging systems by antioxidative pathways. The salt-tolerant plant presented lower increase in the amounts of ROS and antioxidant enzymes than the salt-sensitive plants. Therefore, the increment in ROS such as H_2O_2 content and antioxidant enzymes activity in leaves depended upon the levels of salt tolerance (Dubey, 1994; Zhu, 2001; Parida and Das, 2004; Slesak et al., 2007). Chen et al. (1993) and Duand (1997) proposed that peroxidase and catalase are two major systems for the enzymatic removal of H_2O_2 in plants. Until now, the information about the biochemical parameters of each plant that can grow on high salinity soil is insufficient. Therefore, in this study, we use H_2O_2 and peroxidase enzyme as potential indicators to detect the ability of plants to grow on high salinity areas.

METHODOLOGY

Soil and plant samples were collected in May, 2010 at the area near Akkrasathsoonthorn Reservoir, Somsanuk Village, Borabue District, Mahasarakam Province, where the high salinity of soil has been improved by tree plantation during the past 2 years. The study area at the beginning was classified into high salinity (6.23 dS m^{-1}) and very high salinity (18.9 dS m^{-1}) areas. After 2 years of tree plantation, the salinity of both areas decreased to slight salinity ($< 1 \text{ dS m}^{-1}$).

Electrical conductivity and pH of soil were measured by using a conductivity meter and a pH meter, respectively, based on a soil-water ratio of 1:5 by weighing 10 g of soil in a beaker. The soil sample was left for 30 minutes to measure the electrical conductivity and for an hour to measure pH value.

Hydrogen peroxide content in the leaves was determined according to the method of Sergiev et al. (1997). Leaf tissues (0.5 g) were homogenized with 5 ml of 0.1% (w/v) trichloroacetic acid (TCA) on an ice bath. Crude extract was centrifuged at $12,000 \times g$ for 15 min and 0.5 ml of the supernatant was transferred to a 15 ml test tube. The supernatant was added with 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0), 1 ml of 1 M KI, and mixed by vortexing briefly. The absorbance of mixture was read at 390 nm. A mixture without the supernatant served as a blank. The content of H_2O_2 was calculated from a standard curve and the concentration was expressed as $\mu\text{mole g}^{-1}$ fresh weight.

Peroxidase activity was determined using the guaiacol oxidation method (Chance and Maehly, 1955) in a 3 ml reaction mixture containing 10 mM phosphate buffer (pH 6.4), 8 mM guaiacol, 100-200 μl crude extract and 2.75 mM H_2O_2 . The increase in absorbance was recorded at 470 nm within 30 s (linear phase) after H_2O_2 was added. One unit of peroxidase activity was expressed as $\Delta A_{470} \text{ min}^{-1} \text{ mg protein}^{-1}$.

Soluble protein content of the crude extract was determined according to the method of Bradford (1976) using the Bio-Rad assay kit (Bio-Rad Laboratories, USA) with bovine serum albumin (BSA) as a calibration standard.

RESULTS AND DISCUSSION

In the present study, the H_2O_2 content and the peroxidase activity in the leaves of 6 plant species harvested from various salinity areas were measured. With increasing EC value in soil, salinity had a significant effect on the amounts of H_2O_2 and peroxidase enzyme activity in all 6 cultivated plant species. The data show that the leaf H_2O_2 contents in 6 cultivated plant species were increased and different (Fig. 1).

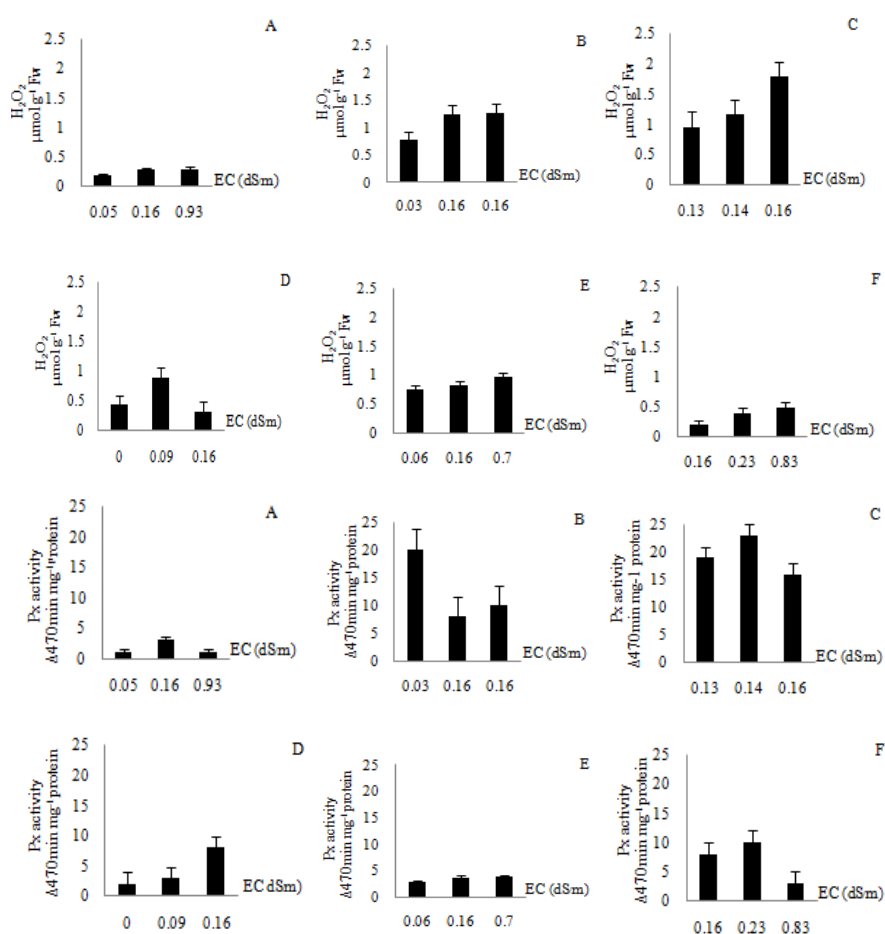


Fig. 1 The effects of salinity of the soil on hydrogen peroxide content and peroxidase activity in leaves of 6 plants.

Combretum quadrangulare (A), *Pithecellobium dulce* (B), *Albizia lebbek* (C), *Acacia auriculiformis* (D), *Cassia siamea* (E), *Acacia magium* (F)

With the increase of EC value in soil, the relation between salt tolerant plants and the amount of H_2O_2 and peroxidase enzyme activity under salt stress can be classified into 2 groups: group I (less responsive to salt stress than group II) consisting of 4 plant species: *C. quadrangulare*, *A. auriculiformis*, *C. siamea*, and *A. magium*, that showed a slight increase in both the amount of H_2O_2 and the peroxidase activity; and group II (more responsive to salt stress than group I) consisting of *P. dulce* and *Albizia lebbek*, that showed increase in both H_2O_2 content and peroxidase activity in leaves. The result indicated that *C. quadrangulare* is the most tolerant plant based on its ability to

maintain the lowest H_2O_2 content value (ranging from 0.2 - 0.3 $\mu\text{mol/g FW}$) when compared the others. Besides, it showed the lowest peroxidase enzyme activity (ranging from 1-3 units $\text{min}^{-1} \text{mg}^{-1}$ protein). Thus, the ability of salt tolerance in *C. quadrangulare* did not depend on the peroxidase activity. This means it may be equipped with some mechanisms which help protect it from H_2O_2 .

These results support previous reports such as Fadzilla et al. (1997) that described an increase of H_2O_2 production, occurred gradually in response to salt stress in rice plants. In addition, Uchida et al. (2002) reported that the most tolerant cultivar (Pokkali) had a lower level of H_2O_2 than the salt-sensitive Pusa Basmati 1. Also, Vaidyanathan et al. (2003), studied the effect of NaCl stress (100-300 mM) on two rice cultivars differing in salt tolerance. They found that the salt-tolerant Pokkali showed higher activity of catalase and lower levels of H_2O_2 than the salt-sensitive Pusa Basmati 1.

Moreover, in the group I, *C. siamea* also showed a slight increase in both the amount of H_2O_2 (ranging from 0.2 - 0.5 $\mu\text{mol/g FW}$) and the peroxidase activity (ranging from 2 - 4 units $\text{min}^{-1} \text{mg}^{-1}$ protein) with increasing EC value in soil. In contrast, in *A. magium*, the H_2O_2 content and the peroxidase activity increased with salinity levels, but the peroxidase activity decreased at 0.16 dS m^{-1} and 0.93 dS m^{-1} values. The result indicated that it has low efficiency to produce peroxidase activity when it grows on high salinity areas. While, in *A. auriculiformis*, though the H_2O_2 content and the peroxidase activity increased with salinity levels, the H_2O_2 content decreased at 0.16 dS m^{-1} . This means that *A. auriculiformis* has high efficiency to produce antioxidant enzyme to detoxify H_2O_2 . Also, when comparing to the others in group I, *A. auriculiformis* is the best species for growing on high salinity areas.

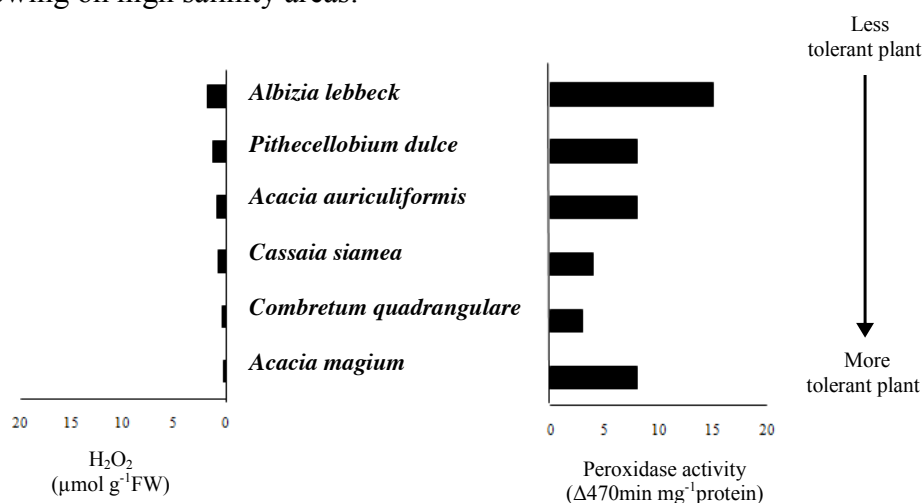


Fig. 2 The effects of salinity of the soil (0.16 dS m^{-1}) on hydrogen peroxide content and peroxidase activity in leaves of 6 plants

When comparing the amount of both parameters at the same electrical conductivity (0.16 dS m^{-1}) in the leaves of 6 plant species (as shown in Fig.2), the amount of H_2O_2 and peroxidase enzyme activity may be depending upon the levels of salinity in soil and also depending on the plant species.

CONCLUSION

The results presented here revealed that salt affected both the content of hydrogen peroxide and the activity of peroxidase in the cultivated plant species. This study confirms that with increasing of EC value in soil, halophytes do not accumulate H_2O_2 uniformly. Both biochemical parameters may be used as potential indicators to detect the ability of plants to survive and grow in their respective areas. This study also indicated that hydrogen peroxide content and peroxidase activity were clearly related to the ability of survival in each plant and the ability to detoxify H_2O_2 , respectively. In summary, the accumulation of both important substances in plants could be used as an index to

indicate the salt tolerance of plant and the best promising species to improve saline soil and to serve as plant energy was *C. quadrangulare*.

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REFERENCES

- Ashraf, M. and Harris, P.J.C. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Science*, 166, 3-16.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254.
- Chance, B. and Maehly, C. 1955. Assay of catalase and peroxidases. *Methods Enzymology*, 11, 764-775.
- Dionisio-Sese, M.L. and Tobita, S. 1998. Antioxidant responses of rice seedlings to salinity stress. *Plant Science*, 135, 1-9.
- Fadzilla, N.M, Finch, R.P. and Burdon, R.H. 1997. Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. *Journal of Experimental Botany*, 48, 325-331.
- Gossett, D.R., Banks, S.W., Millhollon, E.P. and Lucas, M.C. 1996. Antioxidant response to NaCl stress in a control and an NaCl-tolerant cotton cell line grown in the presence of paraquat, buthionine sulfoximine and exogenous glutathione. *Plant Physiology*, 112, 803-809.
- Liu, J. and Shono, M. 1999. Characterization of mitochondria-located small heat shock protein in tomato (*Lycopersicon esculentum*). *Plant and Cell Physiology*, 40, 1297-1304.
- Lin, C.C. and Kao, C.H. 1996. Proline accumulation is associated with inhibition of rice seedling growth caused by NaCl. *Plant Science*, 114, 121-128.
- Mittova, V., Guy, M., Tal, M. and Volokita, M. 2004. Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. *Journal of Experimental Botany*, 55, 1105-1113.
- Munns, R. 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant, Cell and Environment*, 16, 15-24.
- Munns, R., Schachtman, D.P. and Condon, A.G. 1995. The significance of a two-phase growth response to salinity in wheat and barley. *Australian Journal of Plant Physiology*, 22, 561-569.
- Myers, B.A., Neales, T.F. and Jones, M.B. 1990. The influence of salinity on growth, water relations and photosynthesis in *Diplachne fusca* P. Beauv. *Australian Journal of Plant Physiology*, 17(6), 675-691.
- Parida, A.K. and Das, A.B. 2004. Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety*. 1-26 (Article in press).
- Prasad, T.K., Anderson, M.D., Martin, B.A. and Stewart, C.R. 1994. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxidase. *Journal of Plant Physiology*, 6, 65-74.
- Sergiev, I., Alxieva, V. and Karanov, E. 1997. Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. *Comp. Rend. Acad. Bulg. Sci.*, 51, 121-124.
- Slesak, I., Libik, M., Karpinska, B., Miszalski, S. and Miszalski, Z. 2007. The role of hydrogen peroxidase in regulation of plant metabolism and cellular signaling in response to environmental stresses. *Acta Biochem. Pol.*, 54, 39-50.
- Slesak, I., Libik, M. and Miszalski, Z. 2008. The foliar concentration of hydrogen peroxide during salt-induced C3-CAM transition in *Mesembryanthemum crystallinum*. L. *Plant Science*, 174, 221-226.
- Uchida, A., Jagendorf, A.T., Hibino, T., and Takabe, T. 2002. Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Science*, 16, 515-523.
- Vaidyanathan, H., Sivakumar, P., Chakrabarty, R. and Thomas, G. 2003. Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) differential response in salt-tolerant and sensitive varieties. *Plant Science*, 165, 1411-1418.