[Plant Signaling & Behavior 3:3, 199-201; March 2008]; ©2008 Landes Bioscience

Article Addendum

NaCl-induced expression of *ASCORBATE PEROXIDASE 8* in roots of rice (*Oryza sativa* L.) seedlings is not associated with osmotic component

Chwan Yang Hong[1] and Ching Huei Kao[2,*]

[1]Department of Agricultural Chemistry and Institute of Biotechnology; [2]Department of Agronomy; National Taiwan University; Taipei Taiwan, Republic of China

Abbreviations: ABA, abscisic acid; APx, ascorbate peroxidase; ELISA, enzyme-linked immunosorbent assay; Man, mannitol; *OsRab 16A*, the abscisic acid responsive gene; ROS, reactive oxygen species

Key words: abscisic acid, ascorbate peroxidase, *Oryza sativa*, osmotic effect, salt stress

Ascorbate peroxidase (APx; EC 1.11.1.11) plays an important role in scavenging the toxic effects of H_2O_2 in higher plants. Eight types of APx have been described for *Oryza sativa*: two cytosolic (*OsAPx1* and *OsAPx2*), two putative peroxisomal (*OsAPx3* and *OsAPx4*), and four chloroplastic isoforms (*OsAPx5, OsAPx6, OsAPx7 and OsAPx8*). We have recently demonstrated that Na^+ but not Cl^- is required for the NaCl-induced expression of *OsAPx8* in rice roots. Evidence is also provided to show that Na^+-induced expression of *OsAPx8* is mediated through an accumulation of ABA. In addition to its known component of ion toxicity, there is an osmotic effect resulting from salt concentration in the soil. Here we show that ABA level but not *OsAPx8* expression was enhanced at a concentration of mannitol iso-osmotic with 150 mM NaCl suggests that NaCl-enhanced *OsAPx8* expression is not associated with osmotic component.

Soil salinity is one of the major abiotic stresses affecting plant growth and productivity globally. A consequence of salt stress in plants is the excessive generation of ROS.[1,2] Plants have evolved both enzymatic and non-enzymatic mechanisms for ROS scavenging.[3] APx (EC 1.11.1.11) plays a crucial role in the detoxification of cellular H_2O_2, the toxic product of superoxide dismutase.

Expression of *APx* has been reported to be enhanced in plants by NaCl.[2,4-7] In contrast, the expression of *Apx* in sweet potato leaves has been shown to be reduced by NaCl.[8] Moreover, it has been demonstrated that expression of *APx* was not affected by NaCl.[9-11]

The plant hormone ABA is a sesquiterpenoid derived from xanthophyll and appears to influence several physiological and developmental events.[12] The level of ABA in plants increases upon their exposure to NaCl stress.[13-15] It has been shown that ABA treatment increased the expression of *APx* in pea, rice and sweet potato leaves[8,16,17] but had no effect on *APx* gene expression in *Brassica napus*[18] and BY-2 cells.[19]

When growing in saline soil, roots have to cope with two types of stress. The first of these is ionic stress induced by changes in the concentrations of Na^+, Cl^- or both in the root-growing medium and within root tissues. The second is an osmotic stress resulting from salt concentration in the soil that results in lowered water potential and a consequent loss of cell turgor in roots.

Recently, we demonstrated that NaCl-enhanced expression of *OsAPx8* in rice roots is mediated through an accumulation of ABA.[15] Evidence is also provided to show that Na^+ but not Cl^- is required for enhancing *OsAPx8* expression, APx activity and ABA accumulation in rice roots treated with NaCl.[15] To understand the role of osmotic effect in NaCl-induced expression of *OsAPx8*, rice roots were treated with 276 mM mannitol (Man), which is a concentration iso-osmotic with 150 mM NaCl . A key observation of this study is that ABA accumulation but not *OsAPx8* expression was observed in rice roots treated with Man (Fig. 1). In this study, the level of ABA was judged by the transcript level of *OsRab 16A*, an ABA responsive gene[20,21] (Fig. 1). Using ELISA method, Man treatment also resulted in an accumulation of ABA (data not shown) in rice roots. ABA is known to enhance the expression of *OsAPx8* in rice roots.[19] *OsAPx8* is a putative thylakoid isoform.[7] It seems that ABA levels in subcellular compartments of rice roots treated with NaCl differ from those treated with Man. The results obtained so far seem to conclude that NaCl-inducing *OsAPx8* expression in rice roots is associated with ionic but not osmotic component.

*Correspondence to: Ching Huei Kao; Department of Agronomy; NTU; Taipei, Taiwan Republic of China; Tel.: +886.2.3366.4757; Fax: +886.2.2362.0879; Email: kaoch@ntu.edu.tw

Submitted: 12/31/07; Accepted: 01/08/08

Previously published online as a *Plant Signaling & Behavior* E-publication: http://www.landesbioscience.com/journals/psb/article/5541

Addendum to: Chwan Yang Hong, Yi Ting Hsu, Yu Chang, Tsai and Ching Huei Kao. Expression of ASCORBATE PEROXIDASE 8 in Roots of Rice (*Oryza sativa* L.) Seedlings in Response to NaCl. J Exper Bot 2007; 3273–83.

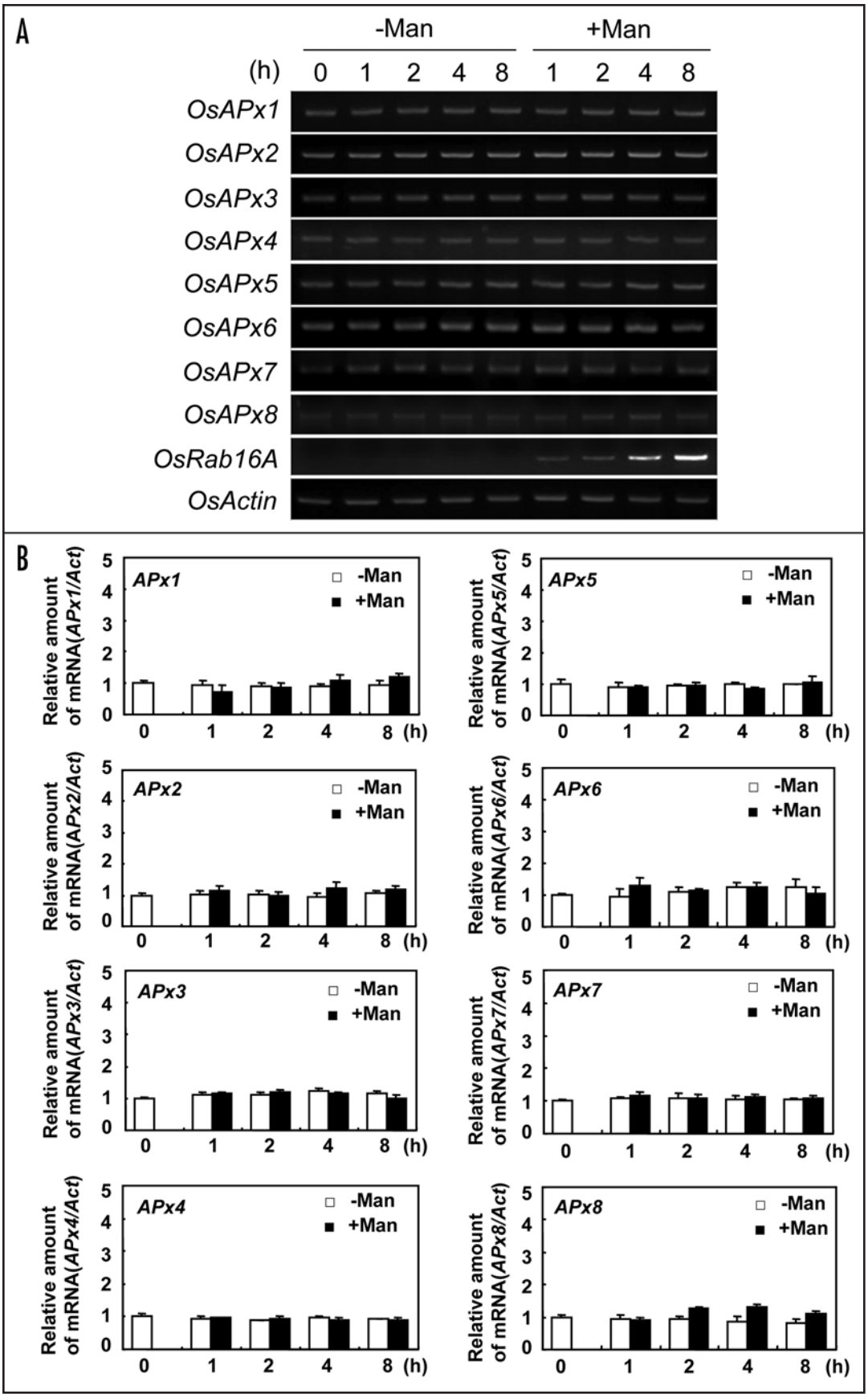

Figure 1. Changes in mRNA levels for *OsAPx* genes and *OsRab 16A* in rice roots in the presence or absence of mannitol (Man). Two-day-old rice seedlings were treated with or without Man (276 mM). Semi-quantitative RT-PCR for *OsAPx* genes and *Rab 16A* was performed. The values of mRNA for the *OsAPx* genes were adjusted by the corresponding amount of *OsActin* mRNA for equality of loading. After the adjustment by *OsActin*, the reaction with the roots without Man was treated as the normalized reference, with a value of one, for determination of the relative amount of mRNA of *OsAPx* genes. Bars show means ± SE (n = 4–6). Statistical differences between measurements on different times were analysed using the LSD test. No significant differences were observed between -Man and +Man at $p > 0.05$.

References

1. Dionisio See ML, Tobita S. Antioxidant responses of rice seedlings to salinity stress. Plant Sci 1998; 135:1-9.
2. Tsai YC, Hong CY, Liu LF, Kao CH. Relative importance of Na^+ and Cl^- in NaCl-induced antioxidant systems in roots of rice seedlings. Physiol Plant 2004; 122:86-94.
3. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 2002; 7:405-10.
4. Hernández JA, Olmos E, Corpas FJ, Sevilla F, Del Rio LA. Salts-induced oxidative stress in chloroplast of pea plants. Plant Sci 1995; 105:151-67.
5. Kawaski S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith D, Bohnert HJ. Gene expression profiles during the initial phased of salt stress in rice. Plant J 2001; 13:889-905.
6. Tsai YC, Hong CY, Liu LF, Kao CH. Expression of ascorbate peroxidase and glutathione reductase in roots of rice seedlings in response to NaCl and H_2O_2. J Plant Physiol 2005; 162:291-9.
7. Teixeira FK, Menezes Benzvente L, Margis R, Margis Pinheiro M. Rice ascorbate peroxidase gene family encodes functionally diverse isoforms localized in different subcellular compartments. Planta 2006; 224:300-14.
8. Park SY, Ryu SH, Jang LC, Kwon SY, Kim JG, Kwak SS. Molecular cloning of a cytosolic ascorbate peroxidase cDNA from cell cultures of sweet potato and its expression in response to stress. Mol Genet Genom 2004; 271:339-46.
9. Lopez F, Vansuyt G, Casse Delbart F, Fourcroy P. Ascorbate peroxidase activity, not the mRNA level, is enhanced in salt-stress *Raphanus sativus* plants. Physiol Plant 1996; 97:13-20.
10. Yoshimura K, Yabuta Y, Ishikawa T, Shigeoka S. Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stress. Plant Physiol 2000; 123:223-34.
11. Menezes Benavente L, Teixeira FK, Kamei CLA, Margis Pinheiro M. Salt stress induces altered expression of genes encoding antioxidant enzymes in seedlings of Brazillian indica rice (*Oryza sativa* L.). Plant Sci 2004; 166:323-31.
12. Seo M, Koshiba T. Complex regulation of ABA biosynthesis. Trend Plant Sci 2002; 7:41-8.
13. Moons A, Bauw G, Prinsen E, Van Montagu M, Van Der Straeten D. Molecular and physiological responses to abscisic acid and salts in roots of salt-sensitive and salt-tolerant indica rice varieties. Plant Physiol 1995; 107:17-22.
14. Montero E, Cabot C, Barcelo J, Poschenrieder C. Endogenous abscisic acid levels are linked to decreased growth of bush bean plants treated with NaCl. Physiol Plant 1997; 101:17-22.
15. Hong CY, Hsu YT, Tsai YC, Kao CH. Expression of *ASCORBATE PEROXIDASE 8* in roots of rice (*Oryza sativa* L.) seedlings in response to NaCl. J Exp Bot 2007; 58:3273-83.
16. Mittler R, Zilinskas BA. Molecular cloning and characterization of gene encoding pea cytosolic ascorbate peroxidase. J Biol Chem 1992; 267:21802-7.
17. Agrawal GK, Jwa NS, Iwahashi H, Rakwal R. Importance of ascorbate peroxidase *OsAPx1* and *OsAPX2 i*n the rice pathogen response pathways and growth and reproduction revealed by their transcriptional profiling. Gene 2003; 322:93-103.
18. Vansuyt G, Lopez F, Inze D, Briat JF, Fourcroy P. Iron triggers a rapid induction of ascorbate peroxidase gene expression in *Brassica nupus*. FEBS Lett 1997; 410:195-200.
19. Bueno P, Riqueras A, Kurapa J, Savoure A, Vebruggen N, Van Montagu M, Inze D. Expression of antioxidant enzymes in response to abscisic acid and high osmoticum in tobacco BY-2 cell cultures. Plant Sci 1998; 138:27-34.
20. Mundy J, Chua HH. Abscisic acid and water-stress induce the expression of a novel rice gene. EMBO J 1988; 7:2279-86.
21. Mundy J, Yamaguchi Shinozaki K, Chua NH. Nuclear proteins bind conserved elements in the abscisic acid-responsive promoter of a rice rab gene. Proc Natl Acad Sci USA 1990; 87:1406-10.