RESEARCH PAPER

Expression of ASCORBATE PEROXIDASE 8 in roots of rice (Oryza sativa L.) seedlings in response to NaCl

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Abstract

Reactive oxygen species are thought to play an important role in NaCl stress. Therefore, the expression patterns of the gene family encoding the H2O2-scavenging enzyme ascorbate peroxidase (APx; EC1.11.1.11) were analysed in roots of etiolated rice (Oryza sativa L.) seedlings in response to NaCl stress. Applying semi-quantitative RT-PCR, the mRNA levels were quantified for two cytosolic (OsAPx1 and OsAPx2), two peroxisomal (OsAPx3 and OsAPx4), and four chloroplastic (OsAPx5, OsAPx6, OsAPx7, and OsAPx8) isoforms identified in the rice genome. NaCl at 150 mM and 200 mM increased the expression of OsAPx8 and the activities of APx, but had no effect on the expression of OsAPx1, OsAPx2, OsAPx3, OsAPx4, OsAPx5, OsAPx6, and OsAPx7 in rice roots. However, NaCl at 300 mM up-regulated OsAPx8 expression, increased APx activity, and down-regulated OsAPx7 expression, but had no effect on the expression of OsAPx1, OsAPx2, OsAPx3, OsAPx4, OsAPx5, OsAPx6, and OsAPx7 in rice roots. The accumulation of abscisic acid (ABA) in response to NaCl was observed in rice roots. Exogenously applied ABA also specifically enhanced the expression of OsAPx8 in rice roots. The accumulation of ABA in rice roots in response to NaCl was inhibited by fluridone (Flu), an inhibitor of carotenoid biosynthesis. Flu treatment also suppressed NaCl-enhanced OsAPx8 expression and APx activity. However, NaCl-enhanced OsAPx8 expression in rice roots is mediated through an accumulation of ABA. Evidence is 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. H2O2 treatment resulted in an enhancement of OsAPx8 induction but no accumulation of ABA. Diphenylene iodonium treatment, which is known to inhibit NaCl-induced accumulation of H2O2 in rice roots, did not suppress OsAPx8 induction and ABA accumulation by NaCl. It appears that H2O2 is not involved in the regulation of NaCl-induced OsAPx8 expression in rice roots.

Key words: Abscisic acid, ascorbate peroxidase, hydrogen peroxide, Oryza sativa, salt stress.

Introduction

Soil salinity, particularly due to NaCl, can be considered as the single most widespread soil toxicity problem that global rice production faces at present. Salinity influences a number of physiological processes. These processes include photosynthesis, nutrient uptake, water absorption, root growth, and cellular metabolism (Werner and Finkelstein, 1995; Hasegawa et al., 2000; Lin and Kao, 2001a, Netondo et al., 2004; Niewiadomska et al., 2004; Chen et al., 2007).

Roots play a number of important roles during plant growth and development, and typically are the first and critical part of the plant to encounter soil salinity. When growing in saline soil, roots have to cope with two types of stress. The first of these 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. The second is ionic stress induced by changes in the concentrations of Na\(^+\), Cl\(^-\), or both in the root growing medium and within root tissues. In addition to its known components of osmotic stress and ion toxicity, salt stress is also manifested as an oxidative stress, all of which contribute to its deleterious effects (Gueta-Dahan et al., 1997; Hernández et al., 2004, 2001; Shalata et al., 2001).

The increase in reactive oxygen species (ROS) seems to occur as a response to most, if not all, abiotic stresses including drought (Smirnoff, 1993) and salinity (Dionisio-Sese and Tobita, 1998; Lin and Kao, 2000; Hernández et al., 2001; Lee et al., 2001; Sudhakar et al., 2001; Hernández and Almansa, 2002; Tsai et al., 2004). To minimize and/or to protect against the toxic effects of these damaging ROS, cells have evolved highly regulated enzymatic and non-enzymatic mechanisms to keep a balance between ROS production and destruction in order to maintain cellular redox homeostasis. ROS-scavenging enzymes include superoxide dismutase, ascorbate peroxidase (APx), glutathione reductase, and catalase (Scandalios, 2002; Mittler et al., 2004).

APx (EC 1.11.1.11) belongs to the class I haem-containing peroxidases found in higher plants (Takeda et al., 1998) and catalyses the conversion of H\(_2\)O\(_2\) to H\(_2\)O and O\(_2\) using ascorbate as the specific electron donor (Asada, 1999). It plays an important role in scavenging and in protecting cells against the toxic effects of H\(_2\)O\(_2\) in higher plants (Shigeoka et al., 1980). The fact that APx has a high affinity for H\(_2\)O\(_2\) and is able to detoxify low concentrations of H\(_2\)O\(_2\), whereas catalase has a high reaction rate but a low affinity for H\(_2\)O\(_2\), renders APx an ideal candidate for tight regulation of H\(_2\)O\(_2\).

APx is located in different cellular compartments. 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) (Teixeira et al., 2004). Using green fluorescent protein–APx fusion proteins in BY-2 cells, Teixeira et al. (2006) observed that OsAPx6 is located in mitochondria, in addition to a chloroplast location.

Expression of APx has been reported to be enhanced in plants by drought and salt (Smirnoff and Combronde, 1988; Mittler and Zilinskas, 1992, 1994; Hernández et al., 1995; Savouré et al., 1999; Sreenivasulu et al., 2000; Kawasaki et al., 2001; Tsai et al., 2004, 2005). In contrast, Park et al. (2004) reported that treatment of sweet potato leaves with NaCl reduced the expression of swAPx1 mRNA. Moreover, it has been demonstrated that the steady-state transcript level of cytosolic APx was not affected by NaCl stress (Lopez et al., 1996; Yoshimura et al., 2000; Menezes-Benavente et al., 2004). Recently, Teixeira et al. (2006) reported that three rice APx genes (OsAPx2, OsAPx7, and OsAPx8) showed altered transcript levels in response to NaCl treatment. The expression of OsAPx2 and OsAPx7 was increased, whereas the OsAPx8 transcript accumulation was strongly suppressed in plants subjected to salt stress (Teixeira et al., 2006).

The plant hormone abscisic acid (ABA) is a sesquiterpenoid derived from xanthophylls (Seo and Koshiba, 2002; Nambara and Marion-Poll, 2005) and appears to influence several physiological and developmental events (Zeevaart and Creelman, 1988; Seo and Koshiba, 2002). It has been shown that ABA accumulates in plants under salt stress (Moons et al., 1995; Montero et al., 1997). Many stress-inducible genes are induced by exogenous ABA treatment. It has been demonstrated that ABA application increased the expression of pea APx1 (Mittler and Zilinskas, 1992), OsAPx1 and OsAPx2 (Agrawal et al., 2003), and swAPx1 (Park et al., 2004), but had no effect on APx gene expression in Brassica napus (Vansuyt et al., 1997) and BY-2 cells (Bueno et al., 1998). Recently, the link between the induction of APx2 expression and leaf water status has been suggested to be mediated by ABA in Arabidopsis (Fryer et al., 2003).

H\(_2\)O\(_2\) is a major ROS generated in plants under stress, which is scavenged by a network of low molecular weight antioxidants and antioxidant enzymes (Asada, 1999). H\(_2\)O\(_2\) has also been implicated in initiating defence responses to a diverse range of biotic and abiotic stresses. It has been shown previously that NaCl treatment increased the H\(_2\)O\(_2\) level in roots of rice seedlings (Lin and Kao, 2001a). H\(_2\)O\(_2\) induced the expression of a gene encoding APx in germinating rice embryos (Morita et al., 1999). However, the failure of H\(_2\)O\(_2\) to induce the APx gene has also been reported (Vansuyt et al., 1997). It has been suggested that cytosolic APx transcripts can be up-regulated by increased levels of H\(_2\)O\(_2\) in tobacco chloroplasts as a result of Cu-Zn-superoxide dismutase overexpression (Gupta et al., 1993). de Agazio and Zacchini (2001) demonstrated that dimethylthiourea, a H\(_2\)O\(_2\) trap, partially prevented the increase of APx gene expression in spermidine-treated maize roots. They concluded that induction of APx gene expression in spermidine-treated maize roots is mediated through H\(_2\)O\(_2\), a spermidine catalytic product. Recent experiments indicate that H\(_2\)O\(_2\) is the principal candidate ROS as a signal involved in the induction of APx2 expression in Arabidopsis leaves by high light stress (Karpinski et al., 1997; Fryer et al., 2003; Chang et al., 2004).

It has been demonstrated previously that OsAPx gene expression was increased in response to NaCl and H\(_2\)O\(_2\) in roots of etiolated rice seedlings (Tsai et al., 2004, 2005). These data were obtained using a non-specific probe, which meant it was not possible to show precisely which member(s) of the OsAPx gene family was induced in response to the NaCl and H\(_2\)O\(_2\) treatments. In this study, using the 3'-untranslated region (UTR)-specific primers
for the OsAPx1, OsAPx2, OsAPx3, OsAPx4, OsAPx5, OsAPx6, OsAPx7, and OsAPx8 genes from rice, the effect of NaCl, ABA, and H₂O₂, on the expression of OsAPx genes was first examined followed by an investigation of whether the induction of OsAPx genes by NaCl is mediated through ABA or H₂O₂.

Materials and methods

Plant material growth conditions

Rice (O. sativa L., cv. Taichung Native 1) seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed extensively with distilled water. In order to obtain more uniformly germinated seeds, rice seeds in a Petri dish (20 cm) containing distilled water were pre-treated at 37 °C for 1 d under dark conditions. Uniformly germinated seeds were then selected and transferred to a Petri dish (9.0 cm) containing two sheets of Whatman No.1 filter paper (Whatman, UK) moistened with 10 ml of distilled water for 2 d. Two-day-old seedlings were then transferred to distilled water, NaCl, ABA, fluridone (Flu), NaNO₃, H₂O₂, and diphenylene iodonium (DPI) at the desired concentration as specified in the individual experiments. Root growth of rice seedlings grown in distilled water is similar to that of those grown in medium containing inorganic salts, thus seedlings grown in distilled water were used as the controls. Each Petri dish contained 20 seedlings and each treatment was replicated four times. The seedlings were allowed to grow at 27 °C in darkness. The same part of the roots of rice seedlings was used for analyses of OsAPx gene expression, APx activity, and ABA level.

Semi-quantitative RT-PCR analysis

Total RNA was isolated from root tissue of 2-d-old etiolated rice seedlings using the TRIZOL reagent (Invitrogen, Carlsbad, CA, USA), according to the supplier’s recommendations. To prevent DNA contamination, RNA was treated with Turbo DNase I (Ambion, Austin, TX, USA) for 30 min at 37 °C before the RT-PCR analysis. The reverse transcription reactions were conducted using the SuperScript III platinum one-step quantitative RT-PCR system (Invitrogen) according to the manufacturer’s protocol.

The gene-specific primers were designed from the 3′–UTR of the OsAPx genes (Teixeira et al., 2006). The sequences used, the predicted amplicons, and the cycle numbers are listed in Table 1. The RT-PCR program initially started with 50 °C/30 min; 94 °C denaturation for 6 min, followed by 94 °C/30 s, and 22–32 cycles of 50 °C/30 s, 68 °C/30 s. The PCRs were optimized for a number of cycles to ensure product intensity within the linear phase of amplification. All tests were repeated at least three times, and one of the repeats is shown in the Results. For all treatments, three replicates of RT-PCR were conducted with three batches of total RNA samples isolated independently. PCR products were resolved by electrophoresis in a 3% agarose gel, stained with ethidium bromide. The gel images were digitally captured with a SynGene gel documentation system and analysed with the GeneTools analysis software (Syngene, Frederick, MD, USA). The rice OsActin gene was used as a reference for normalization.

Table 1. Primers used in semi-quantitative RT-PCR assay

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence (5′ to 3′)</th>
<th>Products (bp)</th>
<th>Cycles</th>
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<td>OsAPx1</td>
<td>APx1-5′</td>
<td>TAGTCTACTACTGTCTAGTAC</td>
<td>160</td>
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<tr>
<td></td>
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<td>25</td>
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<tr>
<td>OsAPx3</td>
<td>APx3-5′</td>
<td>GCACTGACACGACATT</td>
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<td>28</td>
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<tr>
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<td>APx3-3′</td>
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<td></td>
<td>APx5-3′</td>
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<td>APx8-3′</td>
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<td>245</td>
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<td>OsActin</td>
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<td>Actin-3′</td>
<td>ATGCTCTTCCCTCATGATC</td>
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loss was <3% by the method described here. ABA content is expressed on the basis of dry weight.

Statistical analysis
Statistical differences between measurements (n=4–6) on different treatments or on different times were analysed using the LSD test.

Results

NaCl induces OsAPx8 expression and APx activity
In a previous work, it was shown that increasing concentrations of NaCl from 50 mM to 150 mM progressively increased APx activity (Tsai et al., 2004). In the present study, 2-d-old rice seedlings were treated with 150, 200, and 300 mM NaCl for 8 h. The activity of APx of NaCl-stressed rice roots was higher than that of control (Fig. 1B). However, the increase in APx activities was higher in rice roots treated with 150 mM NaCl than in those treated with 200 mM and 300 mM NaCl (Fig. 1B). To investigate the effect of different concentrations of NaCl on the expression of all eight OsAPx genes in rice roots, the total RNA was extracted and the expression dynamics of eight OsAPx genes in rice roots, the total RNA was extracted and the expression dynamics of eight OsAPx genes was examined by semi-quantitative RT-PCR analysis. After 8 h treatment with NaCl (150, 200, and 300 mM), the OsAPx8 transcript was specifically increased (~2- and 3-fold) (Fig. 1A). Figure 1A also shows that the OsAPx8 expression in rice roots induced by 200 mM and 300 mM NaCl was less than that induced by 150 mM NaCl. However, no significant increase due to NaCl (150, 200, and 300 mM) could be detected in the expression of OsAPx1, OsAPx2, OsAPx3, OsAPx4, OsAPx5, and OsAPx6 (Fig. 1A). The expression of OsAPx7 was not affected by 150 mM and 200 mM NaCl, but was decreased (~40%) by 300 mM NaCl (Fig. 1A).

When 2-d-old seedlings were subjected to 150 mM NaCl for 0.5, 1, 2, and 4 h, it was observed that the OsAPx8 transcript was specifically increased (~2-fold) after 1 h treatment with NaCl (Fig. 2A). However, no significant increase due to NaCl could be detected in the expression of OsAPx1, OsAPx2, OsAPx3, OsAPx4, OsAPx5, OsAPx6, and OsAPx7 (Fig. 2A).

NaCl increases ABA level
It has been shown that ABA accumulates in plant tissues in response to salt stress (Moons et al., 1995; Montero et al., 1997). To understand if NaCl treatment results in accumulation of ABA in roots of rice seedlings, the level of ABA in rice roots was determined by ELISA. When 2-d-old rice seedlings were treated with 150 mM NaCl, the level of ABA in roots increased rapidly and peaked...
2 h after NaCl treatment, and then declined (Fig. 2B). The increase in ABA level due to NaCl (0.5 h after treatment) was observed to occur prior to the induction in OsAPx8 expression (1 h after treatment) (Fig. 2A, B).

**Exogenous application of ABA induces OsAPx8 expression**

To test whether ABA is involved in the regulation of OsAPx genes, the effect of 9 μM ABA on the expression of OsAPx genes was examined. It was observed that OsAPx8 mRNA was significantly increased by ABA after 0.5 h of treatment in comparison with the control (Fig. 3A). However, ABA treatment had no effect on the expression of OsAPx1, OsAPx2, OsAPx3, OsAPx4, OsAPx5, OsAPx6, and OsAPx7 (Fig. 3A). Figure 3B also shows that the increase in ABA level could be detected at 0.5 h after ABA treatment.

**Fluridone effect**

The role of ABA in NaCl-enhanced expression of the OsAPx8 gene was tested further by using Flu, which is known to inhibit the conversion of phytoene to phytofluene in the carotenoid biosynthesis pathway (Kowalczyk-Schröder and Sandmann, 1992). The data revealed that NaCl-enhanced ABA accumulation in rice roots was significantly reduced by Flu pre-treatment (Fig. 4C). NaCl-enhanced OsAPx8 expression and APx activity in rice roots was also observed to be suppressed by Flu (Fig. 4A, B). The effect of Flu on the expression of OsAPx8 and APx activity can be reversed by the application of ABA (Fig. 4A, B).

**Na+ but not Cl− is required for increasing OsAPx8 expression, APx activity, and ABA level**

To test whether Cl− is involved in enhancing the expression of OsAPx8, experiments were performed to compare the effect of NaCl (150 mM) with that of NaNO3 (150 mM). The effect of NaNO3 and NaCl on the expression of OsAPx8, APx activity, and ABA level is shown in Fig. 5A–C. Clearly, OsAPx8 transcript, APx activity, and ABA level in roots treated with NaNO3 are similar to those in roots treated with NaCl.

**NaCl-induced OsAPx8 expression is not controlled by H2O2**

The effect of 10 mM H2O2 on the expression of the OsAPx genes is shown in Fig. 5A. H2O2 treatment had no effect on the expression of the OsAPx1, OsAPx2, OsAPx3,
OsAPx4, OsAPx5, OsAPx6, and OsAPx7 in rice roots. In contrast, H$_2$O$_2$ significantly increased the expression of OsAPx8. H$_2$O$_2$ treatment enhanced the expression of OsAPx8 in rice roots at about the same magnitude (2-fold increase) as NaCl treatment (Fig. 6A). However, NaCl, but not H$_2$O$_2$, increased the ABA level in rice roots (Fig. 6B). In the present study, it was also observed that 0.1 µM DPI pre-treatment had no effect on the expression of OsAPx8 (Fig. 7A) and the level of ABA (Fig. 7B) in NaCl-treated rice roots.

Discussion

There are eight APx genes in rice (Morita et al., 1997, 1999; Agrawal et al., 2003; Teixeira et al., 2004, 2006). Here, it is shown that the transcripts of eight OsAPx genes were detectable in roots of 2-d-old etiolated rice seedlings (Figs 1A, 2A). The expression profile of individual APx genes of plants in response to NaCl has been reported (Savoure´ et al., 1999; Menezes-Benavente et al., 2004; Park et al., 2004). Teixeira et al. (2006) were the first to conduct a systematic study of the expression patterns of OsAPx genes in response to NaCl. In their experiments, 2-week-old greenhouse-grown rice plants (cv. Taim7) were treated with 250 mM NaCl for a much longer time (24–96 h). They demonstrated that the expression of OsAPx2 and OsAPx7 increased during NaCl treatment, whereas the expression of OsAPx8 was drastically down-regulated by NaCl stress. In the present study, 2-d-old etiolated rice seedlings (cv. Taichung Native 1) were exposed to 150–300 mM NaCl for 8 h. It is shown that OsAPx8 expression in rice roots was specifically enhanced by all concentrations of NaCl tested and OsAPx7 expression was down-regulated by 300 mM NaCl (Fig. 1A). Thus, the discrepancy in the regulation of the OsAPx genes of rice plants in response to NaCl between the present results and the results of Teixeira’s group is unlikely to be due to different NaCl concentrations, but is more probably due to differences in cultivars, plant age, organs, and growing conditions.

The level of ABA in plants increases upon their exposure to environmental stress (Zeevaart and Creelman, 1988), such as drought (Tian et al., 2004) and salinity (Moons et al., 1995; Montero et al., 1997). Here, it is shown that ABA accumulation in rice roots was induced by NaCl stress (Fig. 2B). It is now well established that ABA in higher plants is derived from C$_{40}$-carotenoids (Seo and Koshiba, 2002; Nambara and Marion-Poll, 2005). As Flu is an inhibitor of ABA biosynthesis through the

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**Fig. 3.** Changes in mRNA levels of OsAPx genes (A) and ABA levels (B) in rice roots in the presence or absence of abscisic acid (ABA). Two-day-old rice seedlings were treated with distilled water or ABA (9 M). Semi-quantitative RT-PCR for OsAPx genes was performed as described in Materials and methods. 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 ABA was treated as the normalized reference, with a value of one, for determination of the relative amount of mRNA. Bars show means ±SE (n=4–6). * and ** represent values that are significantly different between – ABA and + ABA treatments at P<0.05 and 0.01, respectively.
carotenoid pathway (Kowalczyk-Schröder and Sandmann, 1992), the effects of this inhibitor on the reduction of ABA accumulation in NaCl-treated rice roots (Fig. 4C) may imply that the ABA biosynthetic pathway in response to NaCl appears to be the same as that established in other stress conditions (Zeevaart and Creelman, 1998; Seo and Koshiha, 2002).

It has been shown the ABA application increased the expression of APx genes in pea, rice, and sweet potato (Mittler and Zilinska, 1992; Agrawal et al., 2003; Park et al., 2004), but had no effect on APx gene expression in Brassica napus (Vansuyt et al., 1997) and BY-2 cells (Bueno et al., 1998). Recently, the link between the expression of APx2 and leaf water status has been suggested to be mediated by ABA in Arabidopsis (Fryer et al., 2003). In the present study, it is shown that exogenous ABA specifically induced the expression of OsAPx8 in rice roots (Fig. 3A).

In stress-induced gene expression, ABA has been thought to be a candidate for a signal transducer. The present study indicated that ABA was involved in regulating the expression of OsAPx8 in rice roots by NaCl. This conclusion was based on the following observations: (i) NaCl treatment resulted in an increase in the endogenous level of ABA (Fig. 2B) and the induction of OsAPx8 expression in rice roots (Fig. 2A); (ii) the expression of OsAPx8 in rice roots was enhanced by exogenous ABA (Fig. 3A); (iii) the increase in ABA levels due to NaCl preceded the enhancement of OsAPx8 expression (Fig. 2); (iv) Flu treatment reduced the ABA level, as well as NaCl-induced OsAPx8 expression (Fig. 4); and (v) the effect of Flu on the reduction of OsAPx8 expression caused by NaCl can be reversed by the application of ABA (Fig. 4A). The present results suggest that NaCl-enhanced OsAPx8 expression is mediated through ABA accumulation in rice roots.

In previous work, it was shown that increasing concentrations of NaCl from 50 mM to 150 mM progressively increased both Na⁺ and Cl⁻ levels in roots of rice seedlings (Lin and Kao, 2001b). Of particular interest in the present study are the findings that Na⁺ but not Cl⁻ is required for the NaCl-enhanced expression of OsAPx8, APx activity, and ABA level in rice roots (Fig. 5).

Induction of APx expression by H₂O₂ has been reported before (Karpinski et al., 1999; Morita et al., 1999). In agreement with these findings, OsAPx8 expression in rice roots was enhanced by H₂O₂ (Fig. 5A). Recently, de Pinto et al. (2006) reported that the level and timing of H₂O₂...
production in tobacco BY-2 cells are critical points for APx regulation. The constant production of low amounts of H$_2$O$_2$, which was ineffective in inducing cell death, determines a transient, modest increase in APx activity. DPI is an inhibitor of NADPH oxidases and other flavoenzymes (Cross and Jones, 1986; O’Donnell et al., 1993; Moulton et al., 2000). In previous work, it could be shown that NaCl-induced H$_2$O$_2$ accumulation was significantly inhibited by pre-treatment of rice roots with 0.1 μM DPI (Tsai et al., 2005). This observation has led
to the proposal that NaCl-induced H₂O₂ accumulation may be catalysed by NADPH oxidase (Orozoco-Cárdenas et al., 2001). Here, it is shown that DPI pre-treatment had no effect on the expression of the OsAPx8 and accumulation of ABA in NaCl-treated rice roots (Fig. 7). Based on the present and previous results (Fig. 7; Tsai et al., 2005), it is suggested that OsAPx8 expression and APx activity induced by NaCl are not mediated through H₂O₂ in rice roots. Total root H₂O₂ levels have been measured; however, different activities of antioxidant enzymes could interact in the cell to create local differences in H₂O₂ levels in different cellular compartments and, therefore, the involvement of H₂O₂ in this signalling pathway in rice roots during NaCl stress cannot be excluded. The fact that ABA accumulation was enhanced by NaCl but not by H₂O₂ in rice roots (Figs 2B, 6B) indicates that the signalling pathway for OsAPx8 induction in rice roots by NaCl differs from that by H₂O₂. Ethylene, salicylic acid, and jasmonic acid have also been thought to be candidates for signal transducers. Further work is needed to determine the role of each of these candidates in OsAPx8 gene expression.

A mutant of hexaploid wheat with reduced thylakoid-bound APx (tAPx) has been shown to exhibit impaired electron transport and photosynthetic activity (Danna et al., 2003). Transgenic tobacco plants overexpressing tAPx showed increased tolerance to oxidative stress caused by application of methylviologen and by chilling stress under light conditions (Yabuta et al., 2002). The time-course analyses of NaCl (150 mM) treatment clearly indicated that OsAPx8 expression occurs first (1–4 h after NaCl treatment; Fig. 2A) and then APx activity (8 h after NaCl treatment; Tsai et al., 2005) in rice roots. These results have led to the conclusion that early expression of OsAPx8 during NaCl treatment results in an increase in APx activity in rice roots. In the present study, evidence is also provided to show that the increase in the expression of OsAPx8 is indeed associated with an enhancement in its APx activity (Figs 1, 4, 5). Although OsAPx8 is a putative thylakoid isoform (Teixeira et al., 2004), the present results suggest that OsAPx8 expression by NaCl may affect ROS scavenging properties in rice roots. Clearly, more experiments concerning OsAPx8 knockout mutants and overexpression plants are required for our understanding of OsAPx8 function in rice roots under stress conditions.

**Acknowledgements**

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