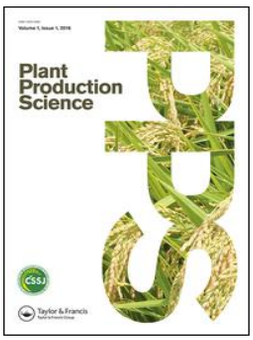

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【Short Report】

Transcription Profiles of Genes Encoding Catalase and Ascorbate Peroxidase in the Rice Leaf Tissues under Salinity

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Abstract: We analyzed the response of transcripts corresponding to ascorbate peroxidase (APX) and catalase (CAT) under salinity in the basal region of the rice leaf, which is tolerant to salinity compared with the apical region. In the NaCl treated plants, the transcript levels of *CATB*, *CATC*, *APX1*, *APX4*, *APX6* and *APX7* increased. The transcript level of *APX2* was comparable to that of the control, but the transcript level of *APX8* was slightly decreased by salinity. The activity of dehydroascorbate reductase decreased by salinity. These results suggest that the increase in CAT activity observed in our previous study is due to the enhancement of transcript levels of *CATB* and *CATC*, and the increase in the transcript level of *APX1*, *APX4*, *APX6* and *APX7* may contribute to maintain APX activity under salinity. The enhancement of the enzyme activities involved in regeneration of ascorbate under salinity is needed to increase APX activity and salinity tolerance in rice plants.

Key words: Ascorbate peroxidase, Catalase, Gene expression, NaCl, Reactive oxygen species, Rice.

The accumulation of excess hydrogen peroxide (H_2O_2) and H_2O_2-derived hydroxyl radical (•OH) are responsible for the deleterious effects of salinity on cellular damage in rice (Yamane et al., 2004). The decrease in the activity of catalase (CAT) by salinity is responsible for the excess generation of H_2O_2 (Yamane et al., 2004). The decrease in CAT activity by salinity is often observed in many kinds of salt-sensitive plants (Corpas et al., 1993; Streb and Feierabend, 1996; Shim et al., 2003). However, we previously showed in a salt-sensitive rice cultivar that CAT activity in the basal region of leaves was increased by salinity, and the activity maintained during the experimental period (Yamane et al., 2009). In addition, the activity of ascorbate peroxidase (APX) in the basal region maintained higher constitutive level under salinity compared with that of the apical region (Yamane et al., 2009). These results suggest that in the basal region of rice leaf, APX and CAT act co-operatively to scavenge H_2O_2 effectively under salinity. However, the gene expression responsible for the increase in CAT activity and the maintenance of APX activity has not been investigated.

Some studies were conducted with rice, focusing on the response to antioxidant enzymes (Shim et al., 2003; Vaidyanathan et al., 2003) and cellular damage (Yamane et al., 2008). However, few studies were conducted on the regulation of antioxidant enzymes at mRNA level. Because, in our previous study, the excess H_2O_2 was responsible for cellular damage of rice leaves (Yamane et al. 2004) and the basal region of the leaf in a salt-sensitive rice cultivar had the ability to remove H_2O_2 effectively (Yamane et al. 2009), we analyzed the transcripts of genes encoding CAT and APX in the basal region of the leaf under salinity. Analysis of the transcripts in the basal region of the leaf could give better insight into the defense mechanisms against salt stress in rice.

Materials and Methods

1. Plant materials and stress treatment

Seeds of rice (*Oryza sativa* L. cv. Nipponbare) were grown hydroponically for 3 wk according to Yamane et al. (2009). The plants were cultured in a growth chamber with 14-hr photoperiod (0800–2200) at 400–500 μmol m^{-2} s^{-1} and 28/20°C (day/night).

The 3-wk-old plants were transferred to 200 mM NaCl in hydroponic culture at 1000. After exposure to NaCl for 0, 24, 48 and 72 hr, 6 cm long segments were sampled from the apical and basal regions of the fully expanded uppermost leaves (6th leaf blades). The leaf segments were immediately frozen with liquid N_2 and preserved at −80°C until use.

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Abbreviations: APX, ascorbate peroxidase; CAT, catalase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; H_2O_2, hydrogen peroxide; MDHA, monodehydroascorbate MDHAR, monodehydroascorbate reductase; •OH, hydroxyl radical; ROS, reactive oxygen species.

Table 1. Primers and probes for detection of *CAT* and *APX* mRNA levels.

Primer	5'-3'	Acc. No.
CATA forward	TCATCTCTTGTTAATTAATTGGAGTACTAC	AK099923
CATA reverse	GAAGTGATAATTTAAATACTTAATAGTAAT	
CATB forward	CCAGTGGTGGTGCTATGTTGGACAGTCAAA	AK100019
CATB reverse	AAACAAGTTCAAACATATAGCACCCACTGT	
CATC forward	GAGATCGATCAGCGTTGCAATCTGCTTCAG	AK066378
CATC reverse	CTTCAGGTTACAGATTATTACATGATGGTG	
APX3 forward	AAGCTATCTGAGCTGGGGTTC	AY382617
APX3 reverse	GCAGACTTAGCAGCACTCACA	
APX5 forward	TCAGCTGCCGATGAACTG	AK073910
APX5 reverse	TGAGG TGTGATGCATCTAATT	
Gene	**Fragment**	**Acc. No.**
APX1	*Pst* Ⅰ	AK061841
APX2	*Pst* Ⅰ /*Bgl* Ⅱ	AK061715
APX4	*Hind* Ⅲ /*Bam*H Ⅰ	AK070842
APX6	*Bgl* Ⅱ /*Xba* Ⅰ	AK061107
APX7	*Bam*H Ⅰ	AK103344
APX8	*Pst* Ⅰ /*Bam*H Ⅰ	AK100016

2. Gene expression

Total RNA was isolated according to Taniguchi et al. (2002) to examine the transcript levels of *CAT* and *APX*. The RNA samples (10 μg) were electrophoresed in a 1.2% agarose gel containing formaldehyde, blotted on a nylon membrane (Hybond-N^+, GE Healthcare), and hybridized with ^{32}P-labeled probes generated from 3'- untranslated region of the full length cDNA clones by polymerase chain reaction and by digesting with restriction enzymes (Teixeira et al., 2006) (Table 1). Equal loading of RNA amount was checked by hybridization with a full-size insert of rice *18S rRNA* (AK059783) (Takaiwa et al., 1984).

3. Activities of dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR)

For the assays, 0.1 g of the leaf segments was homogenated and used. DHAR activity was assayed according to Nakano and Asada (1981) and MDHAR activity according to Hossain et al. (1984). Protein in the supernatant was quantified according to Bradford (1976).

4. Ascorbate content

For the assays, 0.5 g of the leaf segments was homogenated and used. Ascorbate content was measured by the method of Mukherjee and Choudhuri (1983).

5. Statistic analysis

Data were statistically analyzed using ANOVA followed by Tukey's HSD test (SPSS 14.0; SPSS Chicago, IL, USA).

Results

1. Expression of *CAT* genes

No expression of *CATA* was detected in the basal region of the leaf in either the control or NaCl-treated plants (data not shown). Although the expression of *CATB* was not detected in the control plant, the transcript was accumulated after 24 hr of NaCl treatment (Fig. 1A). *CATC* expression in the basal region of the leaf in the control reached a maximum after 48 hr, and was slightly higher in NaCl-treated plants than in the control plants at each harvesting time (Fig. 1B).

No expression of *CATA* was detected in the apical region of the leaf in either the control or NaCl-treated plants (data not shown). While no expression of *CATB* was detected in either the control or NaCl-treated plants, expression of *CATC* transcripts was maximum at 24 hr in the apical region of the leaf in the control (Fig. 1C). The expression of *CATC* decreased after 24 hr of NaCl treatment, and was not detected after 48 hr (Fig. 1C).

2. Expression of *APX* genes

No transcripts of either *APX3* or *APX5* were detected in the basal region of the leaf in either the control or NaCl-treated plants (data not shown). The transcript levels of *APX1*, *APX4*, *APX6* and *APX7* were more than 1.5-fold those in the control after the onset of NaCl treatment (Fig. 2A, C, D, E). Especially, the transcript level of *APX7* drastically increased after 24 hr of NaCl treatment (Fig. 2E). The transcripts of *APX2* and *APX8* were differently modulated under salinity; the transcript level of *APX2* was

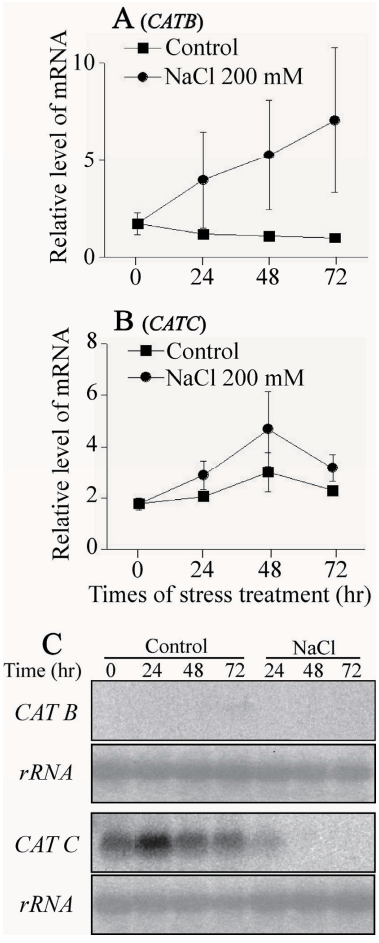

Fig. 1. Changes in the transcript levels of *CAT* in the basal (A, B) and apical (C) regions of the leaf in control and NaCl-treated plants during the experimental period. The transcript levels of *CAT* in the basal region of control (■) and NaCl-treated plants (●) were normalized to the *rRNA* content. Values are means ± SE of 3 individual experiments.

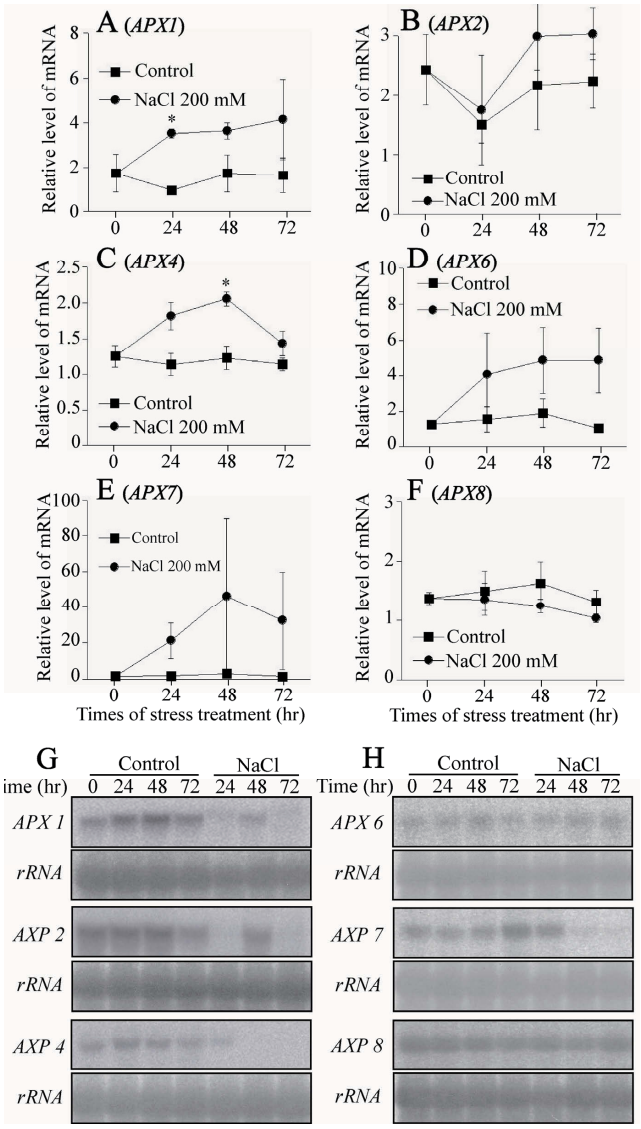

Fig. 2. Changes in the transcript levels of *APX* in the basal (A - F) and apical (G, H) regions of the leaf in control and NaCl-treated plants during the experimental period. See Fig. 1 for detail of the symbols. * above the bars indicates a significant difference from control at $P<0.05$ (Tukey's HSD).

comparable to that in the control (Fig. 2B), while that of *APX8* slightly decreased by salinity (Fig. 2F).

No transcripts of either *APX3* or *APX5* were detected in the apical region of the leaf in either the control or NaCl-treated plants (data not shown). The transcript levels of *APX1*, *2*, *4* and *7* drastically decreased by NaCl treatment (Fig. 2G, H). The transcripts of *APX6* and *APX8* in the apical region of the leaf in the NaCl-treated plants were comparable to that of the control during salt stress (Fig. 2H).

3. DHAR and MDHAR activities and ascorbate content

Because APX is labile in the absence of ascorbate, the system for regeneration of ascorbate constituted by DHAR and MDHAR is essential (Asada, 1999). MDHAR activity in the basal region of the leaf in NaCl-treated plants was comparable to that in the control during the experimental period (Fig. 3B). MDHAR activity in the apical region of the leaf in the NaCl-treated plants increased after 24 hr of NaCl treatment, and then decreased after 48 hr of NaCl treatment (Fig. 3B). The activity of DHAR decreased immediately by salinity in both apical and basal regions (Fig. 3A).

Non-enzymatic antioxidant ascorbate decreased considerably in both apical and basal regions of the leaf compared with that of the control after NaCl treatment (Fig. 3C). Especially, no ascorbate was detected in the apical region of NaCl-treated plants after 48 hr of NaCl treatment (Fig. 3C).

Discussion

Rice contains three *CAT* genes (*CATA*, *CATB* and *CATC*). In rice plants, the expression of *CATB* has been detected in roots but not in leaves (Iwamoto et al., 2000). In the present study, however, the transcript was detected in

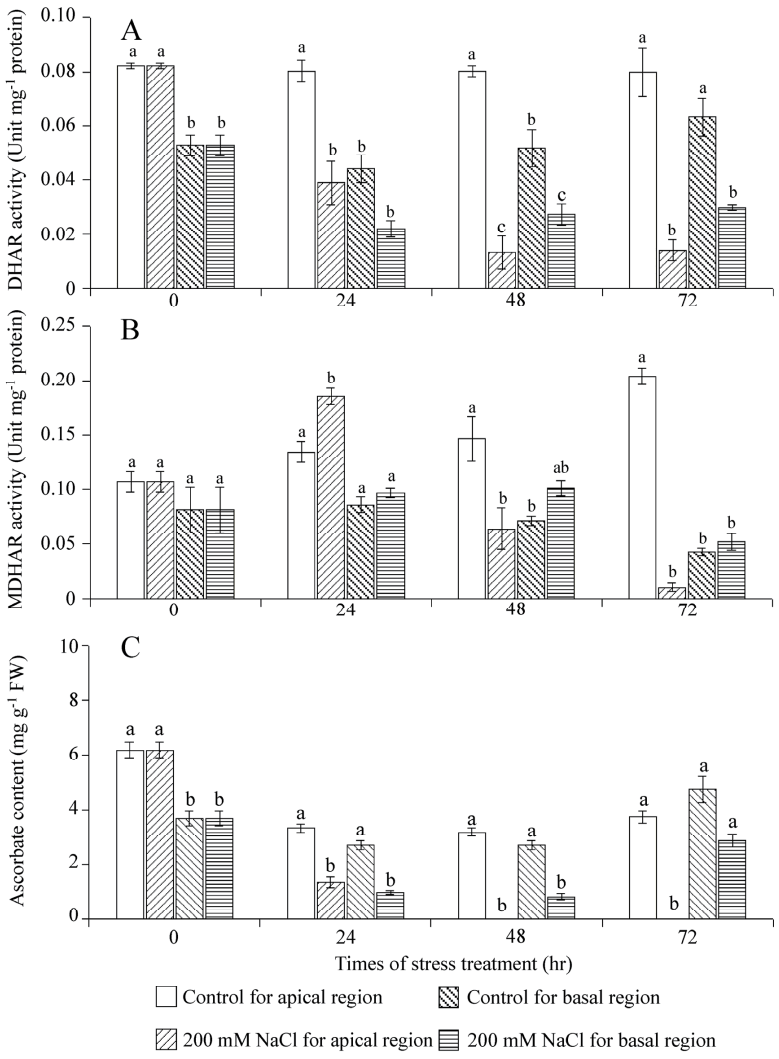

Fig. 3. Changes in the activities of DHAR (A) and MDHAR (B) and ascorbate content (C) in the apical and basal regions of the leaf in control and NaCl-treated plants. Values are means ± SE of 4 replicates. Different letters above the bars indicate a significant difference at $P < 0.05$ (Tukey's HSD).

leaves under salinity (Fig. 1A). The expression of *Nicotiana plumbaginifolia CAT2*, which is homolog to rice *CATB*, is also induced by salinity (Savouré et al., 1999). Iwamoto et al. (2004) reported that the *CATB* promoter region between -80 and -73 corresponds to a putative abscisic acid response element in rice. Adaptation of plants to salinity is to a greater extent under transcriptional control - some processes are regulated by ABA (Zhang et al., 2004). These results and the present data suggested that *CATB* is critical for salinity tolerance. In addition, the transcript level of *CATC* slightly increased under salinity (Fig. 1B). *CATC* has been considered critical for stress tolerance (Kozaki and Takeba, 1996). Therefore, both CATB and CATC are important for salinity tolerance in rice plants. On the other hand, the expression of *CATC* in the apical region was immediately decreased by salinity, and the transcript of *CATB* was not detected (Fig. 1C). Thus, the apical region of the leaf suffers severer damage than the basal region, and the transcript accumulations of *CATB* and *CATC* under salinity are dependent on tissue age.

The transcript level of *APX4*, which is a putative peroxisomal isoform, increased under salinity (Fig. 2C). The transcript levels of *CATB* and *CATC* also increased under salinity (Fig. 1A, B). Salinity enhances the photorespiration rate, and increases the production of H_2O_2 in peroxisomes (Corpas et al., 1993). APX has much higher affinity for H_2O_2 than CAT (Van Breusegem et al., 2001). The overexpression of peroxisomal isoform *APX3* in tobacco could protect leaves from oxidative stress caused by aminotriazole, which inhibits CAT activity (Wang et al., 1999). These results suggest that APX and CAT act co-operatively to remove H_2O_2 generated in peroxisomes under salinity, and peroxisomal APX could be critical for salt tolerance as well as CAT. On the other hand, the

expressions of *CATB, CATC* and *APX4* in the apical region of the leaf were not detected after 48 hr of NaCl treatment (Figs. 1C, 2G). These results suggest that H_2O_2 generated in peroxisome during photorespiration under salinity can not be scavenged effectively. The decrease in the expression of genes encoding H_2O_2 scavenging enzymes in peroxisome could be one of the reasons why the apical region of the leaf suffers severer damage than the basal region.

The H_2O_2 produced in chloroplasts is scavenged by APX using ascorbate as an electron donor (Asada, 1999). The transcript levels of stromal *APX* genes (*APX6* and *APX7*) increased during NaCl treatment (Fig. 2D, E). An increase in the stromal APX activity could contribute to the adaptation of pea plants to NaCl (Gómez et al., 2004). These results suggest that stromal *APX6* and *APX7* could play a role in the defense mechanism against salt-induced oxidative stress in chloroplasts in rice. Although the transcript accumulations of *APX6* and *APX8* in the apical region of the leaf were comparable to those of the control under salinity (Fig. 2H), the expression of *APX7* was not detected after 48 hr of NaCl treatment (Fig. 2H). In the basal region of the leaf, the transcript level of *APX7* was 10 to 50 times higher as compared with other *APX*s (Fig. 2E). Thus, APX7 is most important iso-enzyme to prevent from salt-induced oxidative damage in chloroplasts.

In our previous study, APX activity in the basal region of the leaf under salinity was not enhanced under salinity compared with that of the control (Yamane et al., 2009), however, the transcript level of APX genes (*APX1*, *APX4*, *APX6* and *APX7*) increased in the present study. APX, especially its chloroplastic isozyme, is labile in the absence of ascorbate (Asada, 1999). Therefore, the system for the regeneration of ascorbate constituted by DHAR and MDHAR is essential in order to enhance APX activity (Asada, 1999). MDHAR activity in the basal region of the leaf was comparable to that of the control (Fig. 3B), but DHAR activity was immediately decreased under salinity (Fig. 3A). In addition, the ascorbate content in the basal region of the leaf decreased considerably compared with that in the control under salinity (Fig. 3C). These results suggest that the decreases in DHAR activity and ascorbate content suppress the increase in APX activity in the basal region of the leaf under salinity, though the transcript levels of *APX1*, *APX4*, *APX6* and *APX7* increased.

In conclusion, the transcript levels of *CATB, CATC*, *APX1*, *APX4*, *APX6* and *APX7* in the basal region of the leaf increased under salinity. An increase in CAT activity observed in our previous study is due to the enhancement of the transcript levels of *CATB* and *CATC*, and the increase in the transcript levels of *APX1*, *4*, *6* and *7* may contribute to maintain APX activity under salinity. The enhancement of DHAR and MDHAR activities and ascorbate content under salinity is needed to enhance the activity of APX and salinity tolerance in rice.

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