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Tissue- and genotype-specific ascorbate peroxidase expression in sweet potato in response to salt stress

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Abstract

The aim of this work was to study the short-term effects of salt stress on the antioxidant system and ascorbate peroxidase (APX) expression in two salt sensitive sweet potato cultivars Tainung 57 (TN57) and Tainung 66 (TN66), and one salt-tolerant cultivar Hsusu 18 (HS18). Plants were grown in plastic pots in a greenhouse for 30 d followed by NaCl treatments (0, 150, 300, and 450 mM) for 0, 24 and 48 h in a growth chamber. Young, fully expanded leaves of each treatment and period of time were clipped for enzyme activity measurements. In addition, different tissues (leaves, stems, and roots) were also harvested to analyze the tissue-specific *APX* gene expression using semiquantitative reverse-transcription polymerase chain reaction (RT-PCR). Three degenerated primers of APX isoforms from cytosol, peroxisomes and chloroplasts were used to amplify the APX complementary DNA of these cultivars. Our results show higher increase in APX activity at 24 and 48 h of salinity (450 mM of NaCl) in salt-stress tolerant genotype than in salt-sensitive ones. The expression of APX isoforms in response to salinity was tissue specific and also dependent on stress duration.

Additional key words: antioxidant enzyme, APX isoforms, *Ipomoea batatas*, RT-PCR.

Introduction

Approximately 20 % of the world's irrigated land is affected by salinity (Munns 2005). Therefore, there is an urgent need to address the problem of salinity, especially with an increasing global population. Plants suffer from composite stresses caused by salinity, including altered nutrient uptake, the accumulation of toxic ions, especially Na^+, osmotic stress, and oxidative stress (Verslues *et al.* 2006). Drastic changes in ion and water homeostasis induced by salinity result in molecular damage, growth arrest, and even cell death (Wang *et al.* 2008). Developing germplasm with increased tolerance to salt stress would be an appropriate solution to this problem. The mechanism of salinity tolerance in plants involves several processes, including osmotic adjustment (ion accumulation and synthesis of compatible solutes) and avoidance of ion toxicity (specific ion metabolism and compartmentalization of toxic ions) (Cheeseman 1988).

Salt stress induces the production of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$). These ROS are necessary for inter- and intracellular signaling, but at high concentrations, they seriously disrupt normal metabolism of plants through oxidation of membrane lipids, proteins, and nucleic acids (Hoque *et al.* 2007). The complex antioxidant defense system includes antioxidant enzymes, such as ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR) (Mittler 2002, Amor *et al.* 2006). The ascorbate-glutathione cycle plays a key role in this defense system, and the components of this cycle are present in different organelles in plant cells (Jimènez *et al.* 1997). Numerous studies have indicated that activities of antioxidant enzymes are correlated with plant tolerance to abiotic stresses, and these enzymes are required to maintain redox homeostasis. High activities of some antioxidant enzymes were found to be important in rice (Prashanth *et al.* 2008), pea (Gomez *et al.* 2004), tobacco (Lee *et al.* 2007), potato (Tang *et al.* 2006), barley (Kim *et al.* 2005), and tomato (He and Zhu 2008, Wang *et al.* 2008) in order to survive oxidative stress after being subjected to different concentrations of NaCl. Nevertheless, studies on the response of the antioxidant

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Abbreviations: APX - ascorbate peroxidase; ROS - reactive oxygen species; RT-PCR - reverse transcription-polymerase chain reaction.

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system of sweet potatoes in terms of their ability to survive salinity stress are scarce. The molecular basis of the salinity stress tolerance of sweet potatoes has not been investigated, even though they are quite tolerant to unfavorable growth conditions. One aspect of the response to stress occurs at the transcriptional level, which involves alteration of the gene expression (Xiong and Zhu 2002, Sairam and Tyagi 2004).

In most higher plants, APX isoforms are encoded by small multigene families, and the different isoforms are distributed in four distinct cellular compartments: chloroplasts (including stroma and thylakoids), microbodies (including glyoxysomes and peroxisomes), mitochondria, and cytosol (Shigeoka *et al.* 2002). Several complementary (c)DNAs for APX were isolated from various plant species such as pea (Mittler and Zilinskas 1994), *Arabidopsis* (Jespersen *et al.* 1997), bell pepper (Schantz *et al.* 1995), spinach (Yoshimura *et al.* 2000), and rice (Teixeria *et al.* 2006).

The effects of NaCl on antioxidant activity of the sweet potato were examined in this study. Induction of antioxidants and osmolytes is part of an integrated strategy for stress defense, and may serve as physiological markers for salinity tolerance traits in sweet potatoes. Further we investigated *APX* genes and their expression in different tissues of sweet potatoes in response to NaCl treatments.

Materials and methods

Plants, cultivation and salt-stress treatments: Three sweet potato [*Ipomoea batatas* (L.) Lam] cultivars, Tainung 57 (TN57), Tainung 66 (TN66), and Hsushu 18 (HS18), were used. HS18 is a salt- and drought-tolerant. Cuttings about 30 cm long were planted in plastic pots containing peat moss, loamy soil, and sand in a ratio of 2:1:1 in October and November 2006 in a greenhouse. They were watered with a half-strength Hoagland solution every other day and the average day/night temperatures were 27/22 °C, respectively, and 11-h photoperiod.

One month later, pots were transferred from the greenhouse to a growth chamber for 5-d acclimation under a 12-h photoperiod (irradiance of 400 μmol m^{-2} s^{-1}) and day/night temperature of 25/21 °C. The control group received only half-strength Hoagland's solution, while the other three groups were watered with this solution supplemented with 150, 300, and 450 mM NaCl, respectively. At 0, 24, and 48 h following salt treatment, leaves, stems, and roots from each plant were clipped and immediately frozen in liquid nitrogen. They were then stored in a -70 °C for subsequent analyses.

Enzyme extraction and activity determination: The cut leaves of each treatment were prepared for SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), APX (EC 1.11.1.11), and GR (EC 1.6.4.2) activity analyses by homogenizing 0.2 g of frozen leaf in 0.99 cm^3 of ice-cold 100 mM HEPES buffer (pH 7.0) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.03 g polyvinylpyrrolidone (PVP). For the APX activity determination, 1 mM ascorbate was added to the homogenization buffer. Extracts were centrifuged at 13 000 *g* and 4 °C for 15 min. The supernatants were then collected in a fresh tube for the enzyme assays. Enzyme activities were determined using a UV/VIS spectrophotometer (*Pharmacia Biotech*, Uppsala, Sweden). CAT activity was assayed by measuring the initial rate of disappearance of H_2O_2 (Hwang and VanToai 1991). CAT assay reaction mixture (2 cm^3) contained 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2, and 0.02 cm^3 of the enzyme extract. The decrease in H_2O_2 was followed by a decline in absorbance at 240 nm (coefficient of absorbance, ε = 40 mM^{-1} cm^{-1}). GR activity was measured by the GSH-dependent oxidation of NADPH. The reaction mixture contained 25 mM Tris-$MgCl_2$ (pH 7.6), 5 mM NADPH, 50 mM GSSG, and 1 cm^3 of the enzyme extract (Foyer *et al.* 1997). The change in absorption at 340 nm (ε = 6220 M^{-1} cm^{-1}) was recorded over 2.5 min. The assay for APX activity was carried out in a reaction mixture containing 50 mM potassium phosphate (pH 7), 0.1 mM EDTA, 1.5 mM ascorbate, 1 mM H_2O_2, and 0.04 cm^3 of the enzyme extract. The change in absorption at 290 nm was recorded 80 s after the addition of H_2O_2 (Nakano and Asada 1981). The concentration of oxidized ascorbate was calculated using ε = 2.8 mM^{-1} cm^{-1}. SOD activity was determined using the SOD assay kit *WST* (*Dojindo Molecular Technology*, Gaithersburg, MD, USA). The SOD assay kit utilizes a *WST* working solution of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-tetrazolium which produces a water-soluble formazan dye upon reduction by superoxide anions. The rate of this reduction was inhibited by SOD and measured at an absorbance of 450 nm.

Determination of the water potential and electrolyte leakage: The water potential (Ψ_w) was measured on the third leaf from the top of each plant using a pressure chamber (*Skyeskpm 1400*, *Plant Water System*, Tokyo, Japan). Electrolyte leakage (EL) of leaves was determined according to the method of Huang and Guo (2005). Leaves were excised and immersed in 15 cm^3 of distilled water in test tubes overnight at room temperature. The initial conductivity of the water was determined using a conductivity meter (*CDM 210*, *Radiometer*, Villeurbanne, France). Tubes were placed in boiling water for 15 min, and then cooled to room temperature. The conductivity was again determined. The relative EL was calculated as a ratio of the conductivity before and after boiling.

Statistical analysis: All data are presented as the mean value of at least three independent sets of experiments

with similar results. Measurements of enzymes were analyzed by a three-factor completely randomized analysis of variance (*ANOVA*) that compared the cultivars, NaCl concentrations, and time periods. For significant values, means were separated by the least significant difference (LSD) test at $P \leq 0.05$, 0.01, or 0.001, using *PC SAS 8.2* (SAS Institute, Cary, NC, USA).

Sequence analysis of APX isoforms: Among antioxidant enzymes, APX activity was most dramatically induced. Therefore, we only investigated temporal and spatial *APX* gene expression profiles. After homologous searches, using the National Center of Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov) with *Entrez* and *BLAST*, sequence alignments for cytosolic APX (cyAPX), peroxisomal APX (pAPX), and chloroplastic APX (chAPX) were constructed with the *Clustal-X* program (Thompson *et al.* 1997). Only open reading frame APX sequences were included in the analysis. Specific primer sets for the APX gene were designed using *Vector NTI* suite *8.0* software with the manufacturer's default criteria (*InforMax*, Frederick, USA).

The degenerate primers of APX were as follows: cyAPX, 5'-GGGAAG(A,C)T(C,G)(A,C)CACAAGG-3' (forward) and 3'-(A,C)AG(G,T)GG(A,G)CGGAAA(A,G)C(A,G)-5' (reverse); pAPX, 5'-GCTTGC(A,C,T)GG(A,T)GT(A,G,T)GT(A,C,T)GC-3' (forward) and 3'-(A,G)(A,C,T)CG(C,G,T)CCTTCC(C,T)T(G,T)GG-5' (reverse); and chAPX 5'-CTTCCCCTGCTGGTCATTTA-3' (forward) and 3'-CTGGCCCATCTTTGGTGTAT-5' (reverse). They were custom synthesized by *Bioengineer Biotech* (Taipei, Taiwan) and were designed to amplify 165-, 98-, and 120-bp regions of the cyAPX, pAPX, and chAPX gene segments, respectively.

RNA extraction and semiquantitative reverse-transcription polymerase chain reaction (RT-PCR) analysis: Total RNA was isolated from 100 mg of leaves, stems, and roots with a *NucleoSpin* RNA plant kit (*Macherey Nagel*, Duren, Germany) and quantified with *GeneQuant* (*Amersham Biosciences*, Buckinghamshire, UK) at 260 nm. First-strand cDNA was synthesized from 1 μg of total RNA using AMV-reverse transcriptase with random hexamers according to the manufacturer's instructions (*Boehringer*, Mannheim, Germany).

The designed APX isoform primers were used for amplification. The PCR was carried out in an *Eppendorf* thermal cycler (Hamburg, Germany): 33, 40 and 33 cycles (94 °C for 15 s), annealing temperature 53, 50 and 54 °C for 30 s, and final extension at 72 °C for 40 s for cyAPX, pAPX, and chAPX, respectively. Various RCP cycle numbers were performed to determine that the exponential phase of amplification was not in the plateau phase, therefore allowing for semiquantitative estimations of transcript levels.

The β-actin gene was used as a control in the semiquantitative RT-PCR with the same conditions as described above. The primers of the actin gene were 5'-GAAAGGAAATACAGTGTCTG-3' (forward) and 3'-CATGACAACAACAAATACAA-5' (reverse) and were designed according to the sweet potato actin sequence from GenBank (accession No. gi59003258).

PCR products were electrophoretically separated on a 1.5 % agarose gel, followed by staining with ethidium bromide, and the predicted sizes of 165 (cyAPX), 93 (pAPX), 120 (chAPX), and 242 bp (β-actin) were verified with a 100-bp DNA marker. RT-PCR for amplifying transcripts of the APX genes was performed at least twice to confirm the accuracy of the results, and one representative result of independent experiments is shown in the figures. The RT-PCR products were then sequenced using the same primer in combination with a *Big Dye* terminator cycle sequencing kit and *ABI Prism 310* genetic analyzer (*Applied Biosystems*, Foster City, CA, USA) to verify that only the transcripts of the corresponding gene were amplified. DNA sequences were compared to sequences deposited in the aforementioned *BLAST* program.

Results

Water potential and electrolyte leakage under salt stress: In all genotypes, the Ψ_w in plants treated with 450 mM NaCl was significantly lower than in control plants or plants treated with 150 mM NaCl for 24 and 48 h. This indicated that high NaCl concentration decreased the tissue water content, subsequently affecting the leaf Ψ_w. Non-significant differences were found between 0 and 48 h in both 0 and 150 mM NaCl-treated plants. Significant decreases in the Ψ_w were observed in all genotypes during 24 h at 450 mM NaCl, and 48 h at both 300 and 450 mM NaCl. HS18 exhibited a significantly higher Ψ_w than both TN57 and TN66 (Table 1).

The percentage of ion leakage is a reflection of the membrane damage. During the time course of salt treatments, both the TN57 and TN66 exhibited higher EL values at 300 and 450 mM NaCl compared to those at 0 and 150 mM NaCl and also higher EL than HS18 under the same treatments (Table 1).

Enzyme analysis under salt stress: APX activity was elevated at 450 mM NaCl treatment lasted for 24 and 48 h similarly in both TN66 and TN57. The APX activity of HS18 plants during salt treatments was considerably higher compared to TN57 and TN66. SOD activities after NaCl application did not significantly differ during the experimental period except for 450 mM NaCl for TN57 and HS18. CAT activity showed no significant differences for all NaCl treatments over time for either TN57 or TN66. For HS18, CAT activity increased at 300 mM NaCl (48 h), and 450 mM NaCl (24 and 48 h). The trend of change in GR activity did not significantly

Table 1. Effects of NaCl at different concentrations on the water potential and electrolyte leakage in leaves of three sweet potato cultivars. Among the three time periods, means with the same lowercase letter do not significantly differ at $P \leq 0.05$. Among the four salt treatments, means with the same capital letter do not significantly differ at $P \leq 0.05$. Each value is the mean of three replicates.

Cultivar	NaCl [mM]	Ψ_w [-MPa] 0 h	24 h	48 h	EL [%] 0 h	24 h	48 h
N57	0	0.67 aA	0.78 bA	0.75 bA	20 aA	22 bA	21 bA
	150	0.66 aA	0.64 bA	0.71 bA	21 aA	18 bA	19 bA
	300	0.69 aB	0.72 bB	1.60 aA	23 aB	26 bB	31 aA
	450	0.78 aB	1.70 aA	1.90 aA	27 aB	29 aB	36 aA
TN66	0	0.57 aA	0.54 bA	0.55 bA	22 aA	22 bA	21 bA
	150	0.61 aA	0.57 bA	0.54 bA	21 aA	23 bA	21 bA
	300	0.59 aB	0.61 bB	2.22 aA	23 aB	24 bB	30 aA
	450	0.60 aB	1.42 aA	2.30 aA	23 aC	25 bBC	35 aA
HS18	0	0.22 bB	0.23 cB	0.23 cB	21 aA	20 bA	18 bA
	150	0.26 bB	0.19 cB	0.25 cB	19 aA	21 bA	19 bA
	300	0.24 bB	0.26 cB	0.96 bA	20 aB	22 bB	24 abB
	450	0.34 bB	0.94 aAB	1.48 abA	22 aC	23 bC	28 aB

Table 2. Effects of NaCl on APX [μmol(H_2O_2) g^{-1}(f.m.) min^{-1}], SOD [U g^{-1}(f.m.) min^{-1}], CAT [μmol(H_2O_2) g^{-1}(f.m.) min^{-1}], and GR [μmol(NADPH) g^{-1}(f.m.) min^{-1}] activities in leaves of three sweet potato cultivars. Among the three time periods, means with the same lowercase letter do not significantly differ at $P \leq 0.05$. Among the four salt treatments, means with the same capital letter do not significantly differ at $P \leq 0.05$. Each value is the mean of three replicates.

Cv.	NaCl [mM]	APX 0 h	24 h	48 h	SOD 0 h	24 h	48 h	CAT 0 h	24 h	48 h	GR 0 h	24 h	48 h
N57	0	11.2 bB	11.8 dB	12.7 eB	1.9 aA	1.9 bA	1.9 bA	76.0 aA	78.3 aA	81.5 aA	0.87 aA	0.76 aA	0.77 bA
	150	11.3 bB	12.4 dB	12.9 eB	1.9 aA	1.8 bA	1.7 bA	78.1 aA	80.1 aA	81.8 aA	0.80 aA	0.82 aA	0.79 bA
	300	13.1 bC	11.2 dC	14.4 eC	2.0 aA	2.1 bA	1.9 bA	79.3 aA	84.1 aA	87.4 aA	0.83 aA	0.79 aA	0.82 bA
	450	12.8 bD	21.8 cC	23.9 cdC	2.1 aB	2.9 aA	3.1 aA	80.9 aA	85.6 aA	89.0 aA	0.81 aA	0.82 aA	0.89 bA
TN66	0	11.0 bB	10.1 dB	10.2 eB	2.0 aA	1.8 bA	2.1 bA	46.2 bB	48.4 bB	53.9 bB	0.89 aA	0.90 aA	0.86 bA
	150	9.6 bB	9.0 dB	10.2 eB	2.3 aA	2.0 bA	2.2 bA	47.1 bB	51.2 bB	53.2 bB	0.93 aA	0.93 aA	0.87 bA
	300	9.2 bC	11.2 dC	18.1 dC	2.2 aA	2.1 bA	1.9 bA	50.8 bB	54.0 bB	56.6 bB	0.92 aA	0.94 aA	0.89 bA
	450	9.8 bD	18.4 cC	19.7 dC	2.2 aB	1.9 bB	1.9 bB	51.6 bB	53.5 bB	60.8 bB	0.98 aA	0.97 aA	1.08 aA
HS18	0	24.1 aA	28.4 bA	29.3 cA	2.1 aA	2.7 bA	2.4 bA	34.7 cC	32.6 cC	36.4 cC	0.59 bB	0.56 bB	0.58 cB
	150	24.7 aA	29.8 bA	30.2 cA	2.5 aA	2.8 bA	2.5 bA	34.0 cC	35.3 cC	39.2 cC	0.55 bB	0.60 bB	0.59 cB
	300	29.4 aB	36.7 bB	68.4 bA	2.4 aA	2.3 bA	2.6 bA	39.1 cC	40.2 cC	60.9 bB	0.53 bB	0.58 bB	0.60 cB
	450	29.8 aB	70.8 aA	80.2 aA	2.5 aB	2.5 bB	3.3 aA	39.8 cC	56.8 bB	68.3 bB	0.54 bB	0.57 bB	0.61 cB

differ in the four NaCl treatments from 0 to 48 h in any plants, with the exception of an elevated value 450 mM NaCl (48 h) for TN66 (Table 2).

Expression of the *APX* gene in different tissues under salt treatments: Semiquantitative RT-PCRs were performed on leaves, stems, and roots of sweet potato cultivars subjected to 450 mM of NaCl. TN57 exhibited a gradual increase in *cyAPX* expression from 0 to 48 h in all tissues (Fig. 1*A*). The expression of *cyAPX* RNA in leaves and roots of TN66 at 48 h were higher than those at 0 h. The *cyAPX* gene transcripts at all times in leaves of HS18 were higher than those of TN66 and TN57, indicating that the activation time of *cyAPX* in HS18 was much shorter than those in TN66 and TN57. The transcript of the *cyAPX* gene in HS18 stems initially increased at 24 h and then began to drop thereafter. The RNA level of gene for β-actin, a housekeeping gene that is consistently expressed in plants, showed stable expressions in all experimental groups (Figs. 1*A*-*C*).

The *pAPX* RNA expression over time differed in the various genotypes and tissues. Increases in pAPX RNA in TN57 and HS18 were apparent in roots and stems at 24 and 48 h (Fig. 1*B*). It was more abundant in TN57 and HS18 than in TN66, indicating that levels of *pAPX* transcripts in TN66 were only slightly modified by NaCl.

The expression of the *chAPX* gene was profoundly induced in all tissues of the various genotypes, especially in TN57 plants (Fig. 1*C*). The *chAPX* expression in roots was increased at 48 h in TN57 may be due to homology basis of chloroplast in leaf and amyloplast in root under RT-PCR using chAPX primers. The *chAPX* expression in root of TN57 was higher than that of TN66 and H18 from 24 to 48 h. There were considerable variations in the

levels of *APX* gene expression in different tissues. In the case of TN66, important increases in transcript levels of *chAPX* in leaves were observed after 24 h of NaCl treatment, which then dropped back to the control level. However, a lower RNA level of *chAPX* in roots of HS18 was found at 24 h compared to 0 and 48 h of NaCl treatment.

Discussion

Salt stress primarily causes osmotic stress and ionic imbalance. The disruption in homeostasis occurs at the tissue level as well as at the whole-plant level (Jithesh *et al.* 2006). In our study, leaf Ψ_w under 450 mM NaCl treatment significantly decreased, indicating that all sweet potatoes cultivars suffered water stress. Ion leakage patterns were similar to those of the Ψ_w. The higher the concentration of NaCl applied the higher was the ion leakage of the corresponding plants. The salt- and drought tolerant cultivar HS18 plants had lower ion leakage and higher Ψ_w under the high NaCl concentration compared to TN57 and TN66 genotypes. Salinity may induce stomatal closure as a consequence of decreased Ψ_w (Melgar *et al*. 2008).

Salinity induced ROS production is well known, so the present work studied changes in enzymes involved in detoxifying ROS in sweet potato in response to salinity stress. Exposure to 450 mM NaCl over time caused significant increases in APX activity in all genotypes. Thus APX was deeply involved in this process and can be used for the rapid monitoring and early detection of salinity stress. High activity of APX in salt-treated plants can reduce the accumulation of H_2O_2 and alleviate damage to membranes. However, homeostasis can be maintained in plant cells by various antioxidant enzymes (Chaparzadeh *et al.* 2004, Wang *et al.* 2008). SOD, GR, and CAT activities were less affected by salinity stress than APX in sweet potato. The results obtained also showed that different genotypes may respond differently to oxidative stress during high-salinity conditions. The salt-tolerant cultivar (HS18) had higher APX activity than the more-sensitive cultivars (TN57 and TN66) (Table 2). Thus enhanced APX activity in HS18 plants promoted salt tolerance as was seen from higher Ψ_w and lower EL in this cultivar (Table 1) under salt stress. A correlation between activities of antioxidant enzymes and salinity tolerance was also demonstrated in rice (Vaidyanathan *et al.* 2003, Demiral and Turkan 2005), foxtail millet (Sreenivasulu *et al.* 2000), tomato (Mittova *et al.* 2002), wheat (Mutlu *et al.* 2009), and lentil (Bandeoglu *et al.* 2004). In addition, it was reported that salt stress led to decreases in the ascorbate-glutathione cycle components in salt-sensitive cultivars and to increases in salt-tolerant ones (Hernàndez *et al.* 2001, Shalata *et al.* 2001, Mittova *et al.* 2004). Gratao *et al.* (2005) showed that responses to oxidative stress induced by biotic and abiotic stresses can vary depending on the plant species, tissue, and length of stress, in addition to other specific aspects. Fedina *et al.* (2009) reported that H_2O_2 pretreatment could directly activate antioxidant enzymes leading to higher resistance to salt stress. H_2O_2-pretreated plants had a better ability to scavenge superoxide radical. Regulation of the synthesis of alternative oxidase (AOX) was also crucial in the tolerance of plants to salt stress (Ferreira *et al.* 2008).

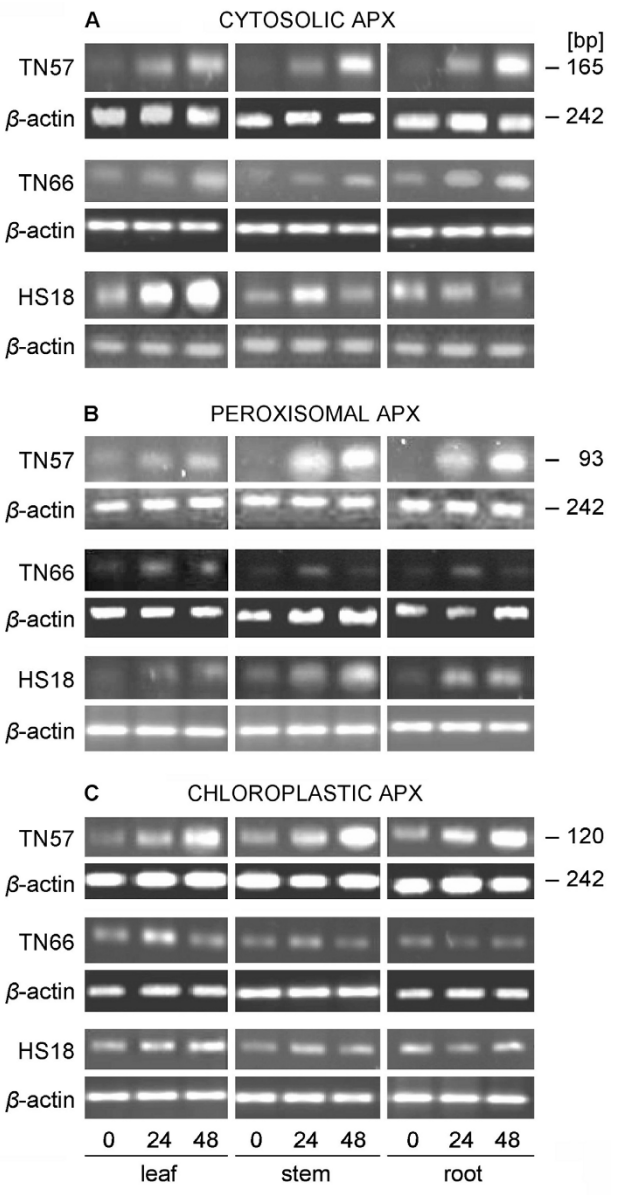

Fig. 1. Semiquantitative RT-PCR of cytosolic (*A*), peroxisomal (*B*) and chloroplastic (*C*) ascorbate peroxidase (*APX*) transcript responses in leaves, stems, and roots of the TN57, TN66 and HS18 genotypes exposed to 450 mM NaCl stress for 0, 24 and 48 h (β-actin was used as the positive control).

Characterization of the isoforms of APX is helpful in elucidating whether or not there are differences among isozymes in their responses to NaCl treatment. The analyses presented here raise many questions about the roles played by the different APX isoforms in antioxidant metabolism in different cell compartments. The APX isoforms of sweet potato genotypes greatly differed in their expression patterns in different plant tissues. Although *APX* transcripts were detected in all tissues, the *pAPX* RNA levels in roots and stems of TN57 and HS18 at 24 and 48 h of salt stress were higher than in TN66 (Fig. 1*B*). In general, increased RNA levels were sustained up to 48 h after the onset of salt treatment in all APX isoforms (Figs. 1*A*-*C*). Similarly, *APX* transcripts in *Arabidopsis* were upregulated in response to salinity, and higher RNA levels were induced by prolonged stress exposures (Attia *et al.* 2008). When plants were exposed to salinity for 24 or 48 h, HS18 plants exhibited a higher *cyAPX* RNA level than did TN57 and TN66 plants (Fig. 1*A*). This result coincided with the markedly increased APX activity in leaves of HS18 plants subjected to 450 mM NaCl for 24 and 48 h (Table 2). In other words, the increase in *cyAPX* RNA levels was related to the maintenance of high APX activity in the cytosol for protecting cellular components against the effects of ROS produced as a consequence of oxidative stress. Increased transcription of the *cyAPX* isoform in response to salt stress (Menezes-Benavente *et al.* 2004) and heat stress (Sato *et al.* 2001) were also reported in rice. However, Yabuta *et al.* (2002) showed that *chAPX* enabled leaf tissues to maintain the photosynthetic activity. When compared to the *cyAPX* and *pAPX* isoforms, *chAPX* was more labile and was rapidly inactivated in the absence of ascorbate (Shigeoka *et al.* 2002). All members of the *APX* gene family, irrespective of the isoforms they encode, are ultimately associated with general cellular metabolism, stress responses, signaling processes, or the development of chloroplasts (Kavitha *et al.* 2008). Plants are adequately protected by the presence of multiple antioxidant enzymes not only in cytosol but also in chloroplasts, mitochondria and peroxisomes. Adjustments of isoform affinities for their substrates may be the main strategy for increasing the efficiency and fine-tuning the antioxidant system (D'Arcy-Lameta *et al.* 2006).

In conclusion, different genotypes of sweet potato displayed variations in their antioxidative systems, and differential *APX* gene expressions in various tissues in each genotype were associated with the salinity-stress response. HS18 plants showed greater resistance to salinity than TN57 and TN66 plants because of increased APX activity and *APX* gene expression. The different APX isoforms functioned in distinct tissues and changed transcription levels in response to salt stress. cyAPX is suggested to play a main role in the defense mechanism against oxidative stress caused by salinity and also might have a function related to stress signaling.

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