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Comparative characterization of sweetpotato antioxidant genes from expressed sequence tags of dehydration-treated fibrous roots under different abiotic stress conditions

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Abstract Drought stress is one of the most adverse conditions for plant growth and productivity. The plant antioxidant system is an important defense mechanism and includes antioxidant enzymes and low-molecular weight antioxidants. Understanding the biochemical and molecular responses to drought is essential for improving plant resistance to water-limited conditions. Previously, we isolated and characterized expressed sequence tags (ESTs) from a full-length enriched cDNA library prepared from fibrous roots of sweetpotato subjected to dehydration stress (Kim et al. in BMB Rep 42:271–276, [5]). In this study, we isolated and characterized 11 sweetpotato antioxidant genes from sweetpotato EST library under various abiotic stress conditions, which included six intracellular CuZn superoxide dismutases (*CuZnSOD*), ascorbate peroxidase, catalase, glutathione peroxidase (*GPX*), glutathione-*S*-transferase, thioredoxin (*TRX*), and five extracellular peroxidase genes. The expression of almost all the antioxidant genes induced under dehydration treatments occurred in leaves, with the exception of extracellular *swPB6*, whereas some antioxidant genes showed increased expression levels in the fibrous roots, such as intracellular *GPX*, *TRX*, extracellular *swPA4*, and *swPB7* genes. During various abiotic stress treatments in leaves, such as exposure to NaCl, cold, and abscisic acid, several intracellular antioxidant genes were strongly expressed compared with the expression of extracellular antioxidant genes. These results indicated that some intracellular antioxidant genes, especially *swAPX1* and *CuZnSOD*, might be specifically involved in important defense mechanisms against oxidative stress induced by various abiotic stresses including dehydration in sweetpotato plants.

Keywords Antioxidant genes · Drought stress · Environmental stress · Expressed sequence tags · Sweetpotato

Abbreviations

ABA	Abscisic acid
APX	Ascorbate peroxidase
CAT	Catalase
DW	Distilled water
EST	Expressed sequence tags
GPX	Glutathione peroxidase
GST	Glutathione-*S*-transferase
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TRX	Thioredoxin

Introduction

Environmental stresses trigger a wide variety of plant responses, ranging from altered gene expression and cellular metabolism to changes in growth rates and crop yields. A plethora of plant reactions exist to circumvent the potentially harmful effects caused by a wide range of both abiotic and biotic stresses, including drought, high salinity, extreme temperatures, high light, and pathogen infections. Drought stress is one of the most adverse environmental stress factors that affects plant growth and productivity [1, 2]. Therefore, understanding the biochemical and molecular responses to drought is essential to improve plant defense mechanisms to

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water-limited conditions. Exposure to drought stress leads to cellular dehydration with concomitant osmotic changes. Removal of water from the cytoplasm into the extracellular space results in a decrease of the cytosolic and vacuolar volumes. Another consequence of exposure to these stresses is the generation of reactive oxygen species (ROS), which in turn have a negative oxidative stress effect on cellular structures and metabolism [2, 3].

As water stresses occur frequently and can affect most habitats, plants have developed several strategies to cope with these challenges. One of the stress defense mechanisms is the antioxidant defense system, which includes antioxidant enzymes and low-molecular weight antioxidants. Particularly, to protect themselves against the toxic ROS that result from stress conditions including dehydration, plants possess very efficient defense mechanisms that include the expression and activation of genes related to intracellular and extracellular ROS scavengers [2, 4].

Sweetpotato (*Ipomoea batatas*) is known as a relatively drought-resistant crop and it represents one of the most globally important root crops grown on marginal land. Although sweetpotato is recognized as a comparatively drought-tolerant plant, the molecular mechanisms underlying this tolerance are not well defined. In a previous study, we isolated and characterized expressed sequence tags (ESTs) from a full-length enriched cDNA library prepared from the fibrous roots of sweetpotato that were subjected to dehydration stress [5]. We performed a functional study of the drought stress-tolerant cytosolic Ib*LEA14* gene isolated from the dehydration stress-treated EST library [6]. Expression analysis showed that dehydration-responsive genes were induced in response to other abiotic stresses, such as NaCl, cold, or ABA treatments. Thus, investigation of dehydration-treated EST pools can provide valuable genetic information about the regulatory networks involved in stress-responsive processes in sweetpotato plants.

In this study, to better understand the adaptability of sweetpotato plants to drought stress in terms of antioxidant defense mechanisms, we isolated and characterized genes for 11 intracellular and extracellular antioxidants from an EST library of dehydration-treated fibrous roots of sweetpotato under various abiotic stress conditions, such as dehydration, salt, cold, and ABA treatments.

Materials and methods

Plant materials

Sweetpotato (*I. batatas* L. Lam. cv. White Star, obtained from Bioenergy Crop Research Center, National Crop Research Institute, RDA, Muan, Jeonnam, Korea) plants were cultivated in a growth chamber in soil at 25 °C under a photoperiod of 16 h light/8 h dark for 50 days.

Analysis of DNA and protein sequences

Sequence identities were determined using the NCBI BLAST search tool, and multiple sequence alignments were performed using the Clustal X and GeneDoc programs. The isoelectric point (pI), molecular weight, and signal sequences of deduced proteins were predicted using the ExPasy (http://www.expasy.org/tools), PSORT (http://psort.ims.u-tokyo.ac.jp), and SoftBerry (http://www.softberry.com) programs.

Stress treatment

Sweetpotato plants grown at 25 °C for 50 days were used for stress treatments. For dehydration treatments, leaves and fibrous roots were collected at 0 (untreated control), 1, 2, 4, 8, 16, and 24 h after treatment. For treatments with NaCl and abscisic acid (ABA), the third leaves from the top were detached from each plant and placed into conical tubes containing 30 ml of sterile water (control), 100 mM NaCl or 0.1 mM ABA, and then incubated at 25 °C for 24 h. For cold stress, plants were exposed to 4 °C (treatment) and 25 °C (control) for 24 h. All treated plant materials were frozen immediately in liquid nitrogen and stored at −70 °C until further use.

Gene expression analysis

Total RNA was isolated from sweetpotato using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and treated extensively with RNase-free DNase I to remove any contaminating genomic DNA. For quantitative expression analysis of antioxidant genes, first strand cDNA was generated from total RNA (2 μg) using MMLV reverse transcriptase (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions. Quantitative RT-PCR was performed in a fluorometric thermal cycler (DNA Engine Opticon 2, MJ Research, Waltham, MA, USA) using EverGreen fluorescent dye, according to the manufacturer's instructions. The inter-experimental quality control comparisons of repeated samples were assessed using CT values between the three replications. A sample that yielded greater than 1.5 of the differential values was removed from the data set. In addition, quality control of the reaction by gel electrophoresis confirmed the presence of a single product of the correct size, and samples with multiple peaks in the dissociation graph were also dismissed to exclude unspecific PCR reactions. Linear data were normalized to the mean CT of α-tubulin as a reference gene, and the relative expression ratio was calculated using

Table 1 Primer sequences used for expression analysis of sweetpotato antioxidant genes in this study

cDNA	Forward primer	Reverse primer	Product size (bp)
swAPX1	TGCCTTCTTTGCTGACTATCCAGA	CGCATACACTTTCAGAGCACAACA	199
CAT	GATTCATCAACCGATGGGTCAAG	CGGTGCCGTTTAATACAACATGC	218
GPX	TGGAATTTCTCCAAGTTCCTTGTTG	ACACAGAGTGCAGCAGCTTCAAA	199
GST	TCAACTACATCCAGTCCTTGGCA	TTTTTGGGACACTTCAACAGGAAA	206
CuZnSOD	TCACCATCACTGATAAGCAGATTCC	TCTGCTAGAAGCGGAGTTTTCACA	198
TRX	TTGCCGTATGATTGCTCCAATACT	CAGATCATCCTTCTTTGCACCCA	190
swPA4	GACGCTTTTCTCGCCGATTT	TTGATGTCGATGGGAACGAA	151
swPA8	CAAGAACATTCTGGAACCCAAGG	GCGACAATTCTTCCTGATCTCTCC	196
swPA9	TCGTACTTCACGCTCATAGCCAA	CGCCGTGCAAACTTTTCTGA	210
swPB6	TTGGACAACAAAGGGTTGATGC	GAGCTTGTTAGCCAGATTGCATTG	201
swPB7	CCGACGCAGCCTTATTAACCAA	TTCAACTGTTCACAACCGCACA	180
swDREB1	TGACGTGGAGCTTGATGCTGAC	GGAATGGACGCTTTTCGCCT	194
IbLEA14	GCCCTGGATGTGGCAGTGAA	GGCAGCTTCTGCCTCTGCTTC	219
α-Tubulin	CAACTACCAGCCACCAACTGT	CAAGATCCTCACGAGCTTCAC	212

the $2^{\text{D-DCT}}$ method. The expression levels of sweetpotato antioxidant genes were analyzed by quantitative RT-PCR using the gene-specific primers listed in Table 1.

Statistical analyses

Data were analyzed by one-way analysis of variance (ANOVA). The subsequent multiple comparisons were examined based on the least significant difference (LSD) test. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 12) and statistical significance was set at $P < 0.05$ and $P < 0.01$.

Results

Isolation and sequence analysis of sweetpotato antioxidant genes

To analyze the antioxidant-related ESTs from the cDNA library of dehydration-treated sweetpotato fibrous roots [5], we grouped the sequences using the National Center for Biotechnology Information (NCBI) database. The classification was based on the best homology match of BLASTX searches against *Arabidopsis* and other plant protein sequences. Eleven antioxidant-related genes were found among the most abundant ESTs (Table 2). The selected clones, each with a query start position upstream of the subject start position in the aligned region, were defined as full-length, except for the catalase (*CAT*) gene that was a partial sequence. Based on the analysis of the 11 selected clones, the average insert size was 638–1,246 bp. Sequence comparison of these clones using BLASTX led to the identification of six known antioxidant-related intracellular genes, CuZn superoxide dismutase (*CuZnSOD*), ascorbate peroxidase (*swAPX1*), catalase (*CAT*), glutathione peroxidase (*GPX*), glutathione-*S*-transferase (*GST*), and thioredoxin (*TRX*), and five extracellular peroxidases (POD), *swPA4*, *swPA8*, *swPA9*, *swPB6*, and *swPB7*. These 11 genes were selected for further characterization.

Differential expression of sweetpotato antioxidant genes in intact tissues

To understand the expression patterns of sweetpotato antioxidant genes, expression of the 11 selected genes in various tissues of untreated plants was examined by RT-PCR analysis (Fig. 1). All 11 genes were highly expressed in fibrous roots. Expression of *CAT* and *TRX* was observed in all tissues, whereas the other genes showed very low transcript levels in leaf tissues. In the storage roots, *CuZnSOD*, *swAPX1*, *CAT*, *TRX*, *swPA8*, *swPB6*, and *swPB7* showed high expression levels under normal culture conditions.

Expression patterns of sweetpotato antioxidant genes in response to drought stresses in leaves and fibrous roots

The expression patterns of the 11 selected genes for intracellular and extracellular antioxidants were investigated by quantitative RT-PCR analysis after dehydration treatments of leaves and fibrous roots. In leaves, expression patterns of six intracellular and four extracellular PODs, *swPA4*, *swPA8*, *swPA9*, and *swPB7*, were induced at 4 or 8 h after dehydration treatment (Fig. 2a). However, the extracellular POD gene *swPB6* showed decreased expression patterns during drought stress. In fibrous roots,

Table 2 Identified antioxidant genes from the EST library of dehydration-treated fibrous roots of sweetpotato plants

Gene name	cDNA size (bp)	GeneBank accession no.	Annotation
Cytosolic ascorbate peroxidase (*swAPX1*)	1,046	AY206407	*Ipomoea batatas* cytosolic swAPX1 (Park et al. 2004)
Peroxisomal catalase (*CAT*)	789	JQ906097	*Ipomoea batatas* peroxisomal CAT (ADB03784.1) *Capsicum annuum* CAT (BAF91369.1)
Cytosolic glutathione peroxidase (*GPX*)	830	JQ906089	*Arabidopsis* AtGPX6 (XP_002874703.1) *Ricinus communis* putative GPX (XP_002509790.1)
Cytosolic glutathione-*S*-transferase (*GST*)	952	JQ906090	*Solanum lycopersicum* putative GST4 (AAG16759.1) *Ricinus communis* putative GST (XP_002532525.1)
Cytosolic CuZn superoxide dismutase (*CuZnSOD*)	841	JQ906095	*Ipomoea batatas* cytosolic CuZnSOD (Q07796.2) *Nicotiana plumbaginifolia* cytosolic CuZnSOD (P27082.2)
Cytosolic thioredoxin (*TRX*)	638	JQ906096	*Ipomoea batatas* cytosolic TRX-H3 (AAQ23135.1) *Nicotiana tabacum* cytosolic TRX-H1 (P29449.1)
Extracellular peroxidase (*swPA4*)	1,283	AY206409	*Ipomoea batatas* extracellular swpa4 (Park et al. 2003)
Extracellular peroxidase (*swPA8*)	1,131	JQ906091	*Arabidopsis* ATP2a peroxidase (AAM65003.1) *Arabidopsis* peroxidase 21 (PER21) (NP_181250.1)
Extracellular peroxidase (*swPA9*)	1,160	JQ906094	*Ricinus communis* peroxidase 27 precursor (XP_002518541.1) *Arabidopsis* peroxidase 27 (PER27) (NP_186768.1)
Extracellular peroxidase (*swPB6*)	1,022	JQ906092	*Nicotiana tabacum* secretory peroxidase (AAD33072.1) *Catharanthus roseus* secretory peroxidase (AAY26520.1)
Extracellular peroxidase (*swPB7*)	1,246	JQ906093	*Ricinus communis* peroxidase 39 precursor (XP_002515748.1) *Arabidopsis* peroxidase RCI3(NP_172018.1)

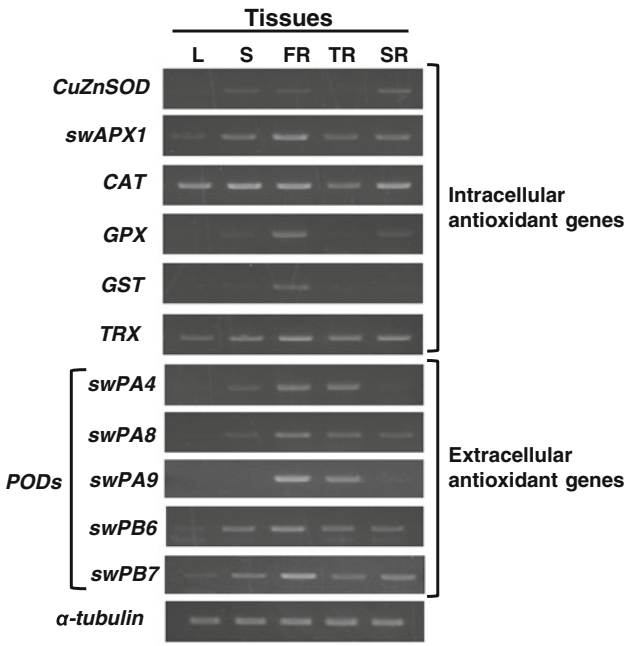

Fig. 1 Expression patterns of genes for intracellular and extracellular antioxidants in different tissues of sweet potato. Total RNAs were extracted from leaves (*L*), stems (*S*), fibrous roots (*FR*), thick roots (*TR*), and storage roots (*SR*). Tubulin was used as a control for equal loading

expression patterns of the intracellular *GPX* and the extracellular POD gene *swPA4* were induced at 8 h after dehydration treatment, whereas expression patterns of the intracellular *TRX* and the extracellular POD gene *swPB7* exhibited early induction by 1 h after dehydration (Fig. 2b). The other genes showed decreased expression patterns during drought stress. The dehydration marker genes *swDREB1* and *IbLEA14* were used as positive controls [6, 7]. Elevated expression of both *swDREB1* and *IbLEA14* were observed 1 h after dehydration treatment in fibrous roots, whereas expression in leaves continued to be up-regulated for 24 h.

Expression patterns of sweetpotato antioxidant genes under salt, cold, and ABA treatments

To investigate the expression of intracellular and extracellular antioxidant genes under various abiotic stresses such as salt, cold, and ABA, quantitative RT-PCR analysis was conducted after treatment of sweetpotato leaves with 100 mM NaCl, 4 °C, and 0.1 mM ABA for 24 h. Expression of six genes for intracellular antioxidants was rapidly induced within 4 h in response to treatment with 100 mM NaCl or 0.1 mM ABA (Fig. 3). Cold treatment at 4 °C for 6 h elicited the induction of five intracellular antioxidant genes, *CuZnSOD*, *swAPX1*, *GPX*, *GST*, and *TRX*, compared with controls. The expression pattern of the *CAT* gene slightly decreased in response to cold treatment at 4 °C.

For the extracellular POD genes, *swPA4*, *swPA8*, *swPA9*, and *swPB7* showed increased expression patterns

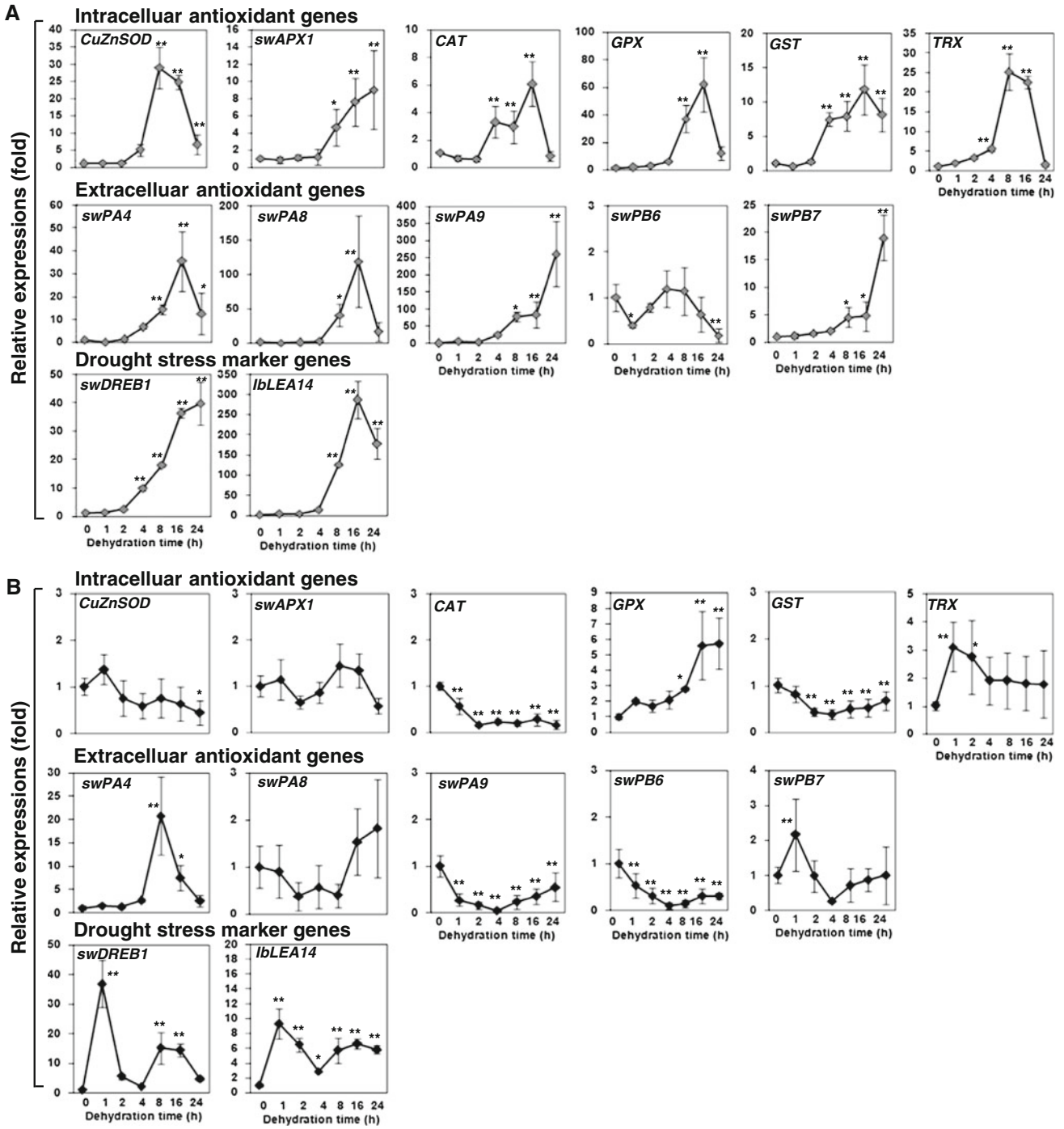

Fig. 2 Expression patterns of genes for intracellular and extracellular antioxidants in leaves and fibrous roots of sweet potato under different dehydration conditions for 24 h. **a** Expression patterns of sweet potato antioxidant genes in leaves under dehydration conditions. **b** Expression patterns of sweet potato antioxidant genes in fibrous roots under dehydration conditions. The *swDREB1* and *IbLEA14* genes were used as dehydration markers. Statistical significance of differences between the control and treatment groups was determined by one-way ANOVA with LSD post hoc test (*$P < 0.05$; **$P < 0.01$)

in response to NaCl, ABA, and cold treatments, whereas *swPB6* showed ABA-specific induction (Fig. 4). Taken together, these data suggest that an ABA-dependent pathway might be involved in the regulation of expression of these 11 antioxidant genes in response to oxidative stress conditions induced by dehydration, salt, and cold.

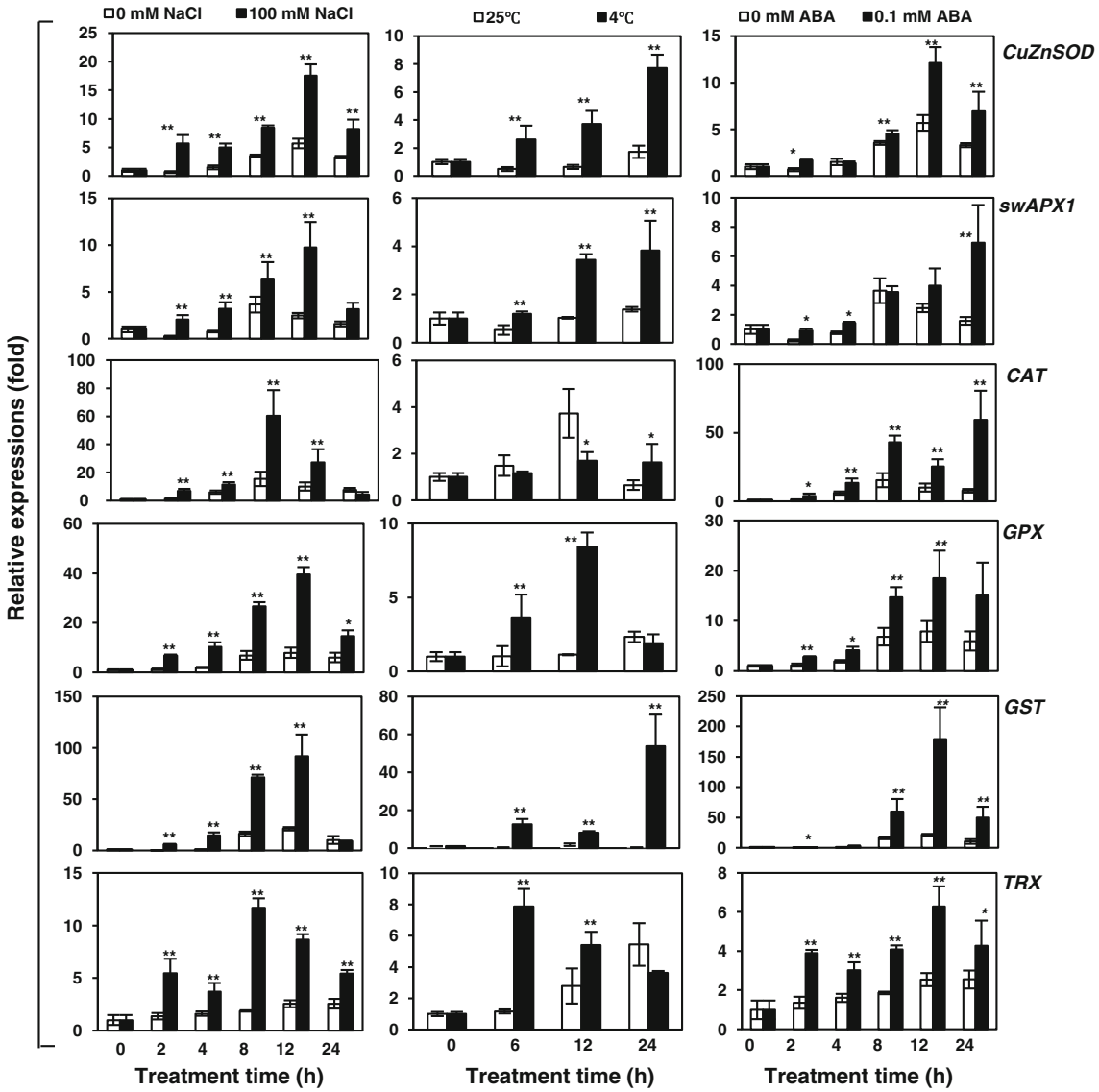

Fig. 3 Expression patterns of intracellular antioxidant genes in sweet potato leaves under different abiotic stress conditions, including 100 mM NaCl, 4 °C, and 0.1 mM ABA for 24 h. Statistical significance of differences between the control and treatment groups was determined by one-way ANOVA with LSD post hoc test (*$P < 0.05$; **$P < 0.01$)

Discussion

Plant drought stress may occur under conditions of reduced soil water content. Gene expression changes in response to drought stress conditions may be regulated directly by the stress conditions or may result from secondary stress responses. Changes in expression of various defense-related genes are regulated by complex plant defense mechanisms. Some plant defense genes are involved in promoting stress tolerance. An important goal is to identify genes that function to promote cellular and whole-plant tolerance of drought stress. Drought stress is exacerbated by the consequent production of ROS in different intracellular and extracellular compartments in plants. Therefore, plant antioxidant genes play important roles in plant defense mechanisms in response to diverse stress conditions, including drought stress. In the current study, we isolated genes for intracellular and extracellular antioxidants from an EST library of dehydration-treated fibrous roots of sweetpotato, and evaluated changes in their expression profiles in plants treated with dehydration, salt, cold, and the phytohormone ABA. Our results showed that the expression of genes for intracellular antioxidants in sweetpotato were more responsive to various abiotic stress conditions than genes for extracellular antioxidants.

Drought stress causes ROS production in different cellular compartments [8]. This enhanced ROS production should be controlled by a versatile and cooperative antioxidant system that modulates intracellular ROS concentrations and sets the redox status of the cell [4]. The first

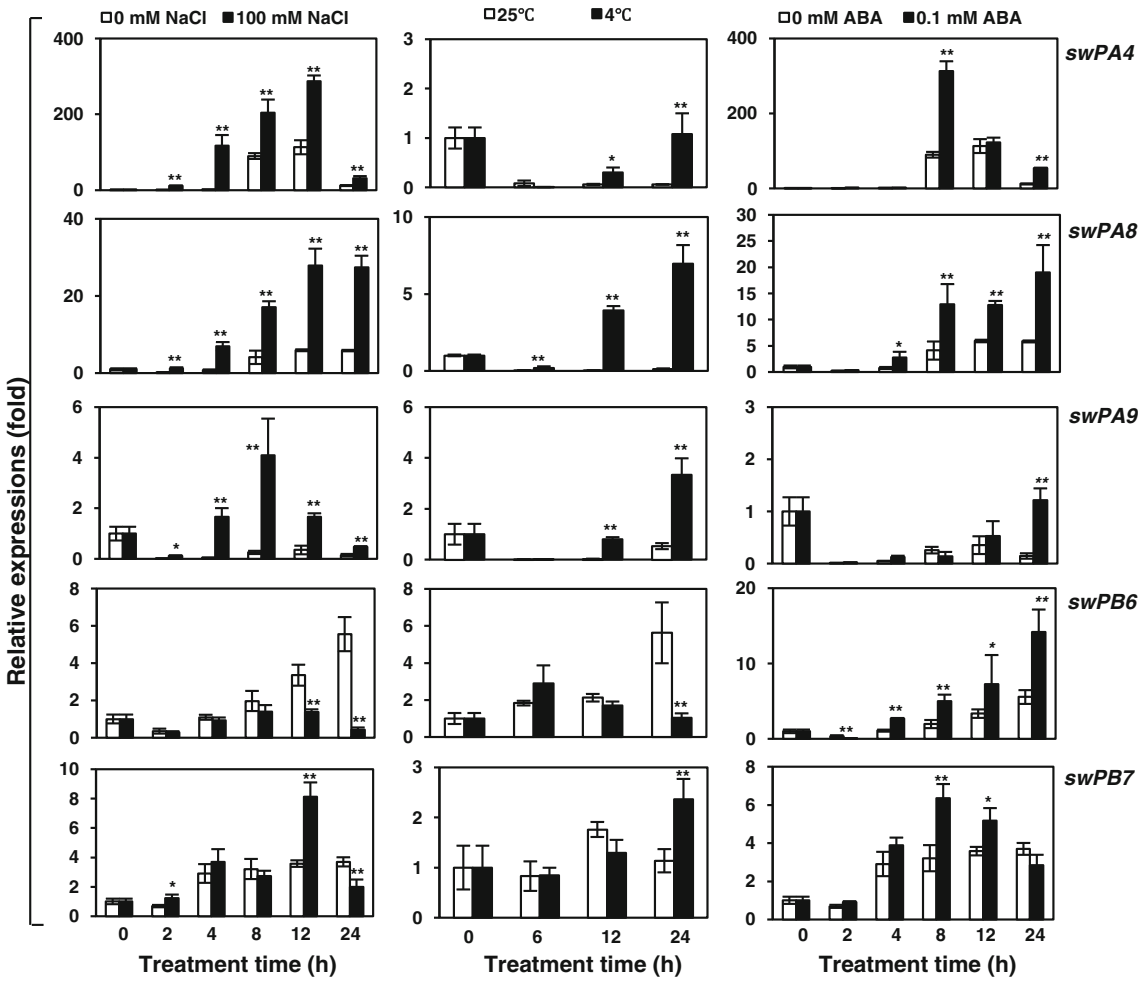

Fig. 4 Expression patterns of extracellular antioxidant genes in sweet potato leaves under different abiotic stress conditions, including 100 mM NaCl, 4 °C, and 0.1 mM ABA for 24 h. Statistical significance of differences between the control and treatment groups was determined by one-way ANOVA with LSD post hoc test (*$P < 0.05$; **$P < 0.01$)

plant organ to detect a limitation of the water supply is the root system, and plant roots also send signals to the leaves through the xylem sap with the phytohormone ABA [9–11]. When the stress signal reaches the leaves during drought stress, it triggers stomatal closure and the plant shifts to a water-saving strategy. Under drought stress, intracellular ROS production is enhanced through multiple pathways and occurs in chloroplasts, mitochondria, peroxisomes, plasma membrane, and cytosol [2, 8]. The major intracellular ROS-scavengers of plants during drought stress include SOD, ascorbate peroxidase (APX), and CAT [2, 4, 8]. The balance between SOD and APX or CAT activities in cells is crucial for determining the steady-state level of *superoxide anionic radicals* ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). This balance, together with sequestration of metal ions, is thought to be important to prevent the formation of the highly toxic hydroxyl radical via the metal-dependent Haber–Weiss reaction or the Fenton reaction [12]. The different affinities of APX (in the μM range) and CAT (in the mM range) for H_2O_2 suggest that they belong to two different classes of H_2O_2-scavenging enzymes. Thus, APX might be responsible for the fine modulation of ROS for signaling, whereas CAT might be responsible for the removal of excess ROS during stress conditions [13, 14]. In this study, dehydration of leaves induced the expression of cytosolic *CuZnSOD*, *swAPX1*, and peroxisomal *CAT* genes (Fig. 2), thereby suggesting that CuZnSOD, APX, and CAT are important for the removal of dehydration-induced ROS in the leaves of sweetpotato plants. GPX is the primary intracellular enzyme capable of repairing membrane lipid peroxidation, and it is generally considered to be the main line of enzymatic defense against oxidative damage in the membrane [15]. In plants, GPX is located mainly in the cytosol and also detoxifies H_2O_2 to H_2O, but uses glutathione (GSH) as a reducing agent [4]. The enhancement of *GST*

expression is considered to be a marker for plant responses to multiple environmental stresses including drought stress [16, 17]. GSTs are a family of multifunctional detoxification enzymes that are mainly located in the cytosol and catalyze the conjugation of a wide variety of xenobiotics to GSH. TRXs are small proteins that function as hydrogen donors and thereby reduce disulfide bridges in proteins. Their involvement in the cellular response to oxidative stress is well documented in various organisms such as animals, yeast, and bacteria [18]. However, the role of TRXs in oxidative stress responses in plants is largely unexplored [3]. In the present study, expression of *GPX* and *TRX* genes was induced by dehydration in leaves and fibrous roots of sweetpotato, whereas expression of *GST* was induced by dehydration only in leaves (Fig. 2). These results suggest that GPX and TRX have important roles in response to dehydration-induced oxidative stress in leaves and fibrous roots of sweetpotato plants.

Cellular drought stress causes changes in cell wall function and characteristics [19], and the accumulation of new proteins in the cell wall played a role in the alteration of cell wall elasticity that occurred throughout the stress periods. A number of defense-related genes that are also induced by drought stress may be suggested to have effects on cell wall characteristics. External stimuli, such as dehydration-induced ROS, contact plant cell walls first, and these effects are mitigated by POD reactions that mediate the removal of ROS. Extracellular PODs are not only involved in scavenging ROS, but they also function in plant growth, development, lignification, suberization, and cross-linking of cell wall compounds [20, 21]. The results of this study showed that the expression of three POD genes (*swPA8*, *swPA9*, and *swPB7*) was induced in response to dehydration only in leaves, whereas a previous report showed high expression of *swPA4* in response to drought stress in leaves and fibrous roots (Fig. 2) [22]. Previous work also reported that the expression of 10 POD genes was induced in response to drought stress in leaves and fibrous roots of sweetpotato plants [22]. During stress conditions, drought-tolerant plants such as common bean, sunflower, and sorghum were shown to have higher POD activity than drought-sensitive plants [23, 24]. Lee et al. [25] reported that elevated POD activity and increased isoenzyme expression were correlated with increased lignin contents under drought stress conditions in white clover. Therefore, it is likely that increased expression of extracellular PODs plays major roles in ROS-scavenging and cell wall-related metabolism during drought stress in sweetpotato.

Drought, salt, and cold stress are known to induce the accumulation of ROS, and these ROS may be signals that elicit ROS scavengers and other protective mechanisms, in addition to acting as damaging agents that contribute to stress injury in plants [26]. The stress-related phytohormone ABA was shown to induce H_2O_2 production [27, 28], suggesting that ROS may be an intermediate signal for ABA signaling that mediates *CAT1* expression [27], thermo-tolerance [29], the activation of Ca_2^+ channels and stomatal closure in guard cells [28, 30], and ABA biosynthesis [31]. The results of the current study showed that the expression of genes for intracellular and extracellular antioxidants was induced by salt, cold, and ABA treatments, except for the *swPB6* POD gene (Figs. 3, 4). Therefore, it is likely that these intracellular and extracellular antioxidant genes from the EST library of dehydration-treated sweetpotato fibrous roots play major roles in ROS-scavenging-related defense mechanisms during various abiotic stresses. The expression levels of the *swPB6* gene were induced only by ABA treatment, whereas it showed decreased expression during drought, salt, and cold treatments, thereby suggesting that the ABA-specific or stress-related function of *swPB6* may differ from the other identified antioxidant genes.

This work employed various abiotic stresses and time-course analyses to investigate stress responses in sweetpotato. Some antioxidant genes exhibited a strong response regardless of the source of stress, whereas others responded only to specific stresses. Thus, the expression levels for the 11 intracellular and extracellular antioxidant genes identified in this study may be classified based on their characteristic responses to drought, salt, cold, and ABA treatment conditions (Fig. 5). We normalized expression levels relative to the highest expression levels observed after exposure to stress. Two genes for intracellular antioxidants, *CuZnSOD* and *swAPX1*, were strongly induced by each of the treatments. Three intracellular antioxidant genes, *GPX*, *TRX*, and *GST*, were strongly induced by three different stress conditions, NaCl, ABA, and dehydration or cold stress. *CAT* expression responded strongly to both NaCl and ABA treatments. The genes for extracellular PODs, *swPB7* and *swPA8*, were strongly expressed in response to three different stress conditions, NaCl, ABA, and dehydration. The gene *swPA9* was expressed only in response to dehydration treatment, whereas *swPB6* responded only to ABA treatment. Expression of *swPA4* responded strongly to both NaCl and ABA treatments. Among the 11 antioxidant genes, 10 are highly responsive to ABA, nine are highly responsive to NaCl, and seven are highly responsive to drought stress. These results suggest that the intracellular and extracellular antioxidant genes characterized in this study responded to various stress conditions via complex signaling pathways. The intracellular antioxidant genes responded more strongly to different stress conditions than the extracellular POD genes. Therefore, during various abiotic stress conditions in sweetpotato, the intracellular antioxidant genes were strongly expressed compared with

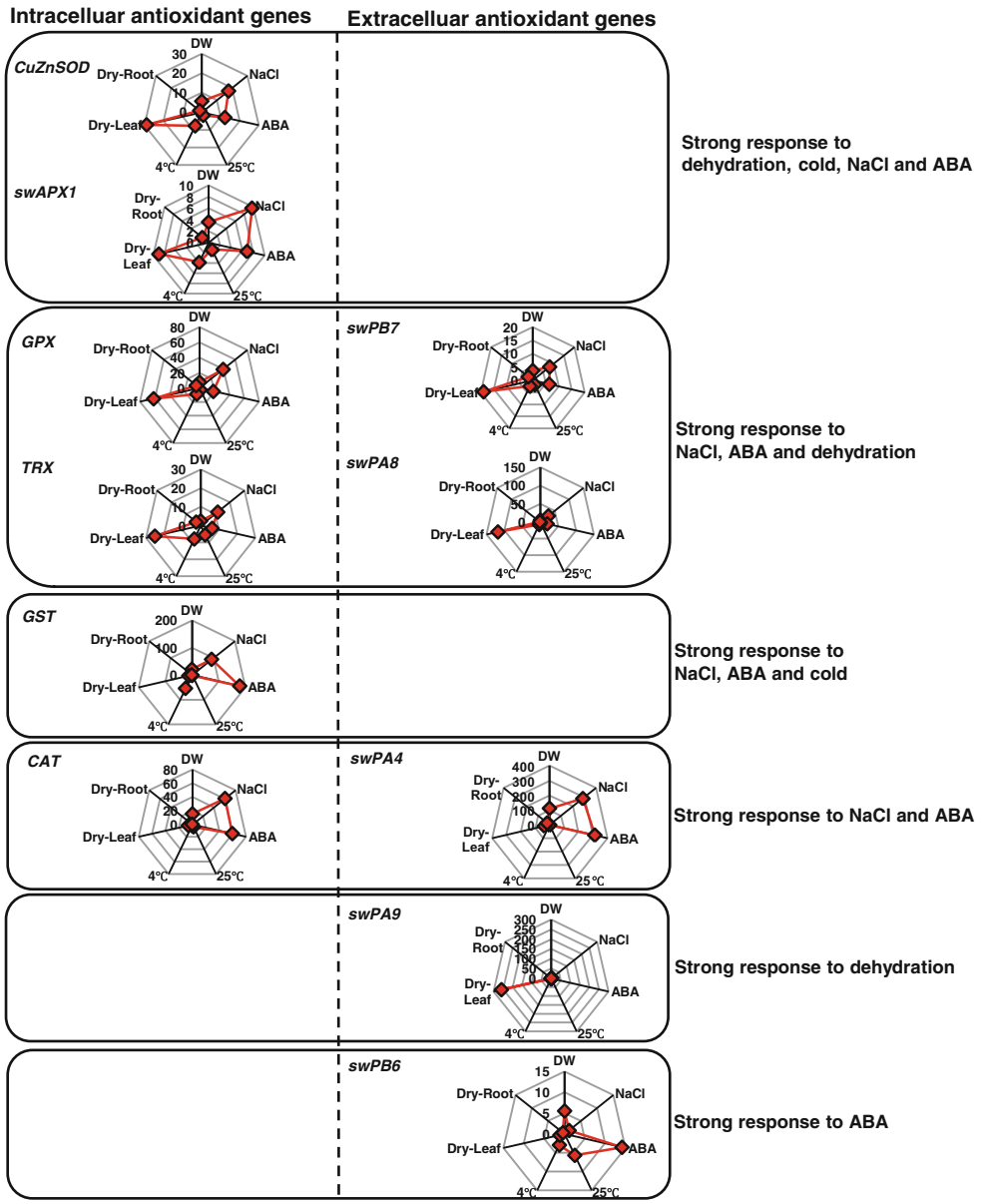

Fig. 5 Summary of relative expression levels of intracellular and extracellular antioxidant genes under normal and abiotic stress conditions. Data indicates that normalized expression levels relative to the highest expression levels observed after exposure to stress. Spider plots of relative expression levels of each antioxidant gene during normal conditions and in response to stress conditions were drawn using Microsoft Excel. The mean measurements from 0 h of normal conditions, represented by control treatments with distilled water (DW) or 25 °C, were assigned a onefold reference value

genes for extracellular antioxidants. The results suggest that *swAPX1* and *CuZnSOD* might have specifically important roles in defense against oxidative stress because of their strong induction in response to various abiotic stresses including dehydration in sweetpotato plants.

In conclusion, this study characterized changes in the expression profiles of 11 genes for intracellular and extracellular antioxidants during various stress conditions in sweetpotato plants. The observed expression patterns of antioxidant genes can provide information on the possible function of each gene during stress conditions in sweetpotato. Changes in the expression levels of antioxidant genes under biotic stress, such as pathogen infection and insect feeding, remain to be studied. Therefore, further studies of antioxidant mechanisms in sweetpotato will be interesting in terms of various biotic stress conditions.

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