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Exogenous salicylic acid enhances wheat drought tolerance by influence on the expression of genes related to ascorbate-glutathione cycle

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Abstract

Treatment with 0.5 mM salicylic acid (SA) significantly alleviated growth inhibition induced by drought in wheat seedlings, manifested by less decreassed fresh mass, dry mass, plant height, root length, and less increased lipid peroxidation. Under drought stress, SA significantly increased the content of ascorbate (ASA) and glutathione (GSH). We determined the full-length cDNA sequences of genes encoding the glutathione-S-transferase 1 (*GST1*) and 2 (*GST2*) and we also measured the transcription of eight genes related to ASA-GSH cycle. The results indicated that exogenous SA significantly enhanced the transcription of *GST1*, *GST2*, glutathione reductase (*GR*), and monodehydroascorbate reductase (*MDHAR*) genes during almost the entire drought period, but only increased those of dehydroascorbate reductase (*DHAR*) at 12 h, glutathione peroxidase (*GPX1*) at 48 h, phospholipid hydroperoxide glutathione peroxidase (*GPX2*) at 12 and 24 h, and glutathione synthetase (*GSHS*) at 12, 24, and 48 h. This implies that SA alleviates the detrimental effects of drought stress on wheat seedling growth by influencing the ASA-GSH cycle.

Additional key words: dehydroascorbate reductase, glutathione reductase, glutathione synthetase, glutathione-S-transferase, monodehydroascorbate reductase.

Introduction

Salicylic acid (SA) is a natural phenolic compound that is essential for establishing the systemic acquired resistance responsible for protecting plants against infections by a broad range of pathogens (Prabhavathi and Rajam 2007, Snyman and Cronjé 2008). SA has received particular attention as it modulates plant responses to several abiotic stressors, such as cold, heat, drought, salt, ultraviolet radiation, and heavy metals (He and Zhu 2008, Ashraf *et al*. 2010, Hayata *et al*. 2010). The majority of these studies have mainly been performed at physiological levels suggesting that SA-induced drought tolerance is associated with an enhanced antioxidant system (Horváth *et al*. 2007a, Mutlus *et al*. 2009, Zhou *et al*. 2009, Hayata *et al*. 2010). However, little information is available about the molecular mechanisms of SA-induced drought or other abiotic tolerances in higher plants, although a few studies have shown that abiotic tolerance induced by SA could be related to the altered expression of the genes encoding osmotin, pathogenesis-related proteins, and heat shock proteins (Ding *et al*. 2002, Kim *et al*. 2002, Clarke *et al*. 2004).

A common effect of abiotic stressors, including drought, is oxidative damage due to a loss of balance between the production and elimination of reactive oxygen species (ROS) (Smirnoff 1998, Li *et al*. 2012). If not effectively and rapidly removed from plants, excessive ROS may damage a wide range of cellular macromolecules such as lipids, proteins, and DNA and ultimately cause cell death. To protect the subcellular components from ROS accumulation, plants respond by inducing antioxidative enzymes, such as superoxide dismutases (SOD), catalase (CAT), peroxidase (POD),

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Abbreviations: ASA - ascorbate; CAT - catalase; DHAR - dehydroascorbate reductase; GPX - glutathione peroxidase; GR - glutathione reductase; GSH - glutathione; GSHS - glutathione synthetase; GST - glutathione-S-transferase; MDA - malondialdehyde; MDHAR - monodehydroascorbate reductase; POX - peroxidase; qPCR - quantitative real-time PCR; RACE - the rapid amplification of cDNA ends; ROS - reactive oxygen species; SA - salicylic acid; SOD - superoxide dismutase.

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and ascorbate-glutathione cycle enzymes [glutathione peroxidase (GPX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR)] as well as non-enzymatic antioxidants, such as glutathione (GSH) and ascorbate (ASA) (Smirnoff 1998, Asada 1999, Mittler 2002, Li *et al*. 2009, Saruhan *et al*. 2012).

Under cold stress, pre-treatment with SA increases ASA and GSH content and induces increased transcription of respective genes in rice and eggplant (Kang and Saltveit 2002, Chen *et al*. 2011). However, we found no reports investigating the relationship between SA, and ASA and GSH biosyntheses under drought stress in higher plants. Wheat is an important drought-sensitive cereal crop whose growth and grain yield are significantly affected by drought stress (Doyle and Fischer 1979, Gao *et al*. 2011). In the present study, we examined the effect of SA application on the transcription of eight genes related to ASA-GSH cycle in wheat seedlings under drought stress to explore the possible molecular mechanisms of SA-induced drought tolerances in this species.

Materials and methods

Seeds of the common wheat (*Triticum aestivum* L.) cv. Yumai 34 were sterilized with 0.01 % (m/v) $HgCl_2$ followed by washing with distilled water. Sterilized seeds were grown hydroponically in full-strength Hoagland's solution (Elberse *et al.* 2003) in glass dishes (diameter 15 cm) in a *FPG-300C-30D* incubator (*Ningbo Laifu Technology Co*., Beijing, China) under a 14-h photoperiod, irradiance of 250 μmol m^{-2} s^{-1}, day/night temperature of 25/15 °C, and relative humidity of 60/75 %. Each dish contained approximately 60 seedlings. After 2 weeks, the seedlings with about three leaves were incubated with fresh Hoagland's medium supplemented with 15 % (m/v) PEG-6000 solution to induce drought stress or in Hoagland's solution supplemented with PEG-6000 solution and 0.5 mM SA (drought stress + SA treatment). As controls, we used those grown only in fresh Hoagland's solution. Growth parameters (plant height, root length, total fresh mass, and total dry mass) were recorded at 72 h after drought treatment in ten wheat seedlings harvested from each treatment. The uppermost fully expanded leaves of seedlings were collected at different time points (0, 12, 24, 48, and 72 h) after initiating the drought stress, immediately frozen in liquid nitrogen, and stored at -80 °C. Lipid peroxidation was determined by estimating malondialdehyde (MDA) content using the method described by Zheng *et al*. (2008). ASA and GSH content was measured according to the methods of Smith (1985) and Kampfenkel *et al*. (1995), respectively.

We found that there were no available reports on the cDNA sequences of the *TaGST1* and *TaGST2* genes coding glutathione-S-transferase in wheat plants. Thus, before measuring the transcript levels of the selected eight genes under drought stress, we isolated the full-length cDNA sequences of the *TaGST1* and *TaGST2* genes using random amplification of cDNA ends (RACE). A *BLAST* search of the wheat expressed sequence tag (EST) databases at *NCBI* (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using the cDNA sequences of the *OsGST1* and *OsGST2* genes in rice (*Oryza sativa*) (GenBank accession Nos. AF402804 and FN428745, respectively) retrieved two wheat EST fragments (CJ949756, HX199872, respectively). These sequences had 89 and 82 % identity to those of the *OsGST1* and *OsGST2* genes, respectively. According to these two EST sequences, primers were designed to amplify the full-length cDNA sequences of the *TaGST1* and *TaGST2* genes using the *BD SMARTTM* RACE cDNA amplification kit (*Clontech*, Palo Alto, CA, USA) according to manufacturer's instructions. The sequence information of the core fragments of the *TaGST1* and *TaGST2* genes was used to design gene-specific primers for the 3′- and 5′-RACE polymerase chain reaction (PCR). The PCR-amplified 3′- and 5′-RACE fragments of both genes were purified and ligated. The full-length cDNA sequences of the *TaGST1* and *TaGST2* genes were obtained by comparing and aligning the sequences of the 3′-RACE and 5′-RACE fragments as well as the EST sequences using *CAP3* software (http://pbil.univ-yon1.fr/lcap3.php). The open reading frame (ORF) regions were amplified by PCR to verify correct alignment. The primers used to amplify the *TaGST1* and *TaGST2* genes are listed in Table 1.

The expression patterns of eight ASA-GSH cycle-related genes were measured in cold-stressed eggplant seedlings (Chen *et al*. 2011). TheY were *GST1*, *GST2*, *GPX1*, *GPX2*, *GR*, *DHAR*, *MDHAR*, and *also GSHS*. The expression patterns of these eight genes were also measured in the present study.

Total RNA was extracted using *Trizol* reagent according to the manufacturer's instructions (*Invitrogen*, Carlsbad, CA, USA). The first-strand cDNA used as template for the RT-PCR was synthesised using a *RevertAid* first-strand cDNA synthesis kit (*Fermentas*, Burlington, ON, Canada) with 2 μg purified RNA. The qPCR was performed using a *SYBR Premix Ex Taq* (perfect real time) kit (*Applied Biosystems*, Foster City, CA, USA) on a *StepOnePLUS* machine according to the manufacturer's instructions. Primers were designed using Primer 5.0 and are listed in Table 2. Each reaction mixture (0.02 cm^3) consisted of 0.01 cm^3 of *SYBR Green Supermix* (2×), 0.001 cm^3 of diluted cDNA, and 0.0005 cm^3 of forward and reserve primers (see Table 2). The PCR cycling conditions were as follows: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 35 s. The relative transcript levels were calculated using the $2^{-\Delta\Delta Ct}$ method with *actin* as the housekeeping gene. To minimize sample variation, mRNA expression of the target gene was normalized relative to *actin* gene expression. Relative gene expression

was calculated as previously described by Livak and Schmittgen (2001) and Chen *et al*. (2011).

The growth experiments were repeated twice with three replicates (nine plants). Gene transcript levels were repeated three times. Each data point was expressed as the average ± SD of three independent replicates. All data were subjected to analysis of variance using *SPSS v. 17.0* software (Chicago, IL, USA) and Duncan's multiple range test ($\alpha = 0.05$) was used to compare the mean values of the different treatments.

Table 1. Primers used to amplify *TaGST1* and *TaGST2* genes using RACE. UPM: the mixture of 0.4 mM long primer 5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3' and 2 mM short primer 5'-CTAATACGACTC-ACTATAGGGC-3'. PCR conditions: a - according to *BD SMARTTMRACE* cDNA amplification kit program; b - cycle parameters were 94 °C for 4 min, 28 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 2 min, and an extension of 72 °C for 8 min; c - cycle parameters were 95 °C for 4 min, 29 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 3 min, and an extension of 72 °C for 6 min. Location indicate the primer position in cDNA, con. means confirmation.

Gene	Method	Primer sequence (5'→3') forward	location	reverse	location	PCR
TaGST1	5' RACE	UPM		CCGAAGAACTTCTTCCCCTCCAGG	447-469	a
	3' RACE	CAAGTGCAACGCCGCCATCTTCCCG	344-368	UPM		a
	RACE con.	AGCACGGAGGTCCAACAAT	13-41	AAGCAGGCGGCTGAAAGAA	808-826	b
TaGST2	5' RACE	UPM		GGCGTGCACCCAGACGACCAGACCG	548-572	a
	3' RACE	ACGCTCGGCGGTCTGGTCGTCTGGG	540-564	UPM		a
	RACE con.	CCGTGCTAGTAGAAAATG	33-50	AGACTCCGAGCTGATGAT	784-801	c

Table 2. DNA sequences of PCR primers used in quantitative real-time PCR determination of eight ASA-GSH cycle-related genes in wheat seedlings.

Gene	Acc. No.	Primer pairs forward	reverse	Expected size [bp]
GST1	JX051003	GCACTGGCCGTCGTTTTACAA	CGGGCCTCTTCGCTATTAC	113
GST2	JX051004	AGCTCTTGGCGTCTTGGCT	AGGCTTCCCCTTGGAGCAC	131
GPX1	AF475124	TGAAGTCTAGCAAAGGTGGCCT	GCGTAGCGATCCACAACAC	97
GPX2	JN578723	CCTAACTAACTCCAACTACACC	TCCTGCCCACCAAACTGAT	105
GR	AY364467	CCTGATGCGGTATTTTCTCCTTA	GCACCATATGCGGTGTGAA	55
DHAR	AY074784	GTGCCTGTGTATAACGGTG	ACAAGTGATGGAGTTGGGT	94
MDHAR	AK371371	AGAAGTTTACGCCCTTCGGC	TTGGAATGTCATCGCCATC	132
GSHS	AJ579382	ATCGCCAAGCTCCGTCAATG	ACAAGTCAGGGTTTTCAATCG	88
Actin	AB181991	AGCGGTCGAACAACTGGTA	AAACGAAGGATAGCATGAGGAAGC	101

Results

Compared to control plants, drought stress (15 % PEG-6000) for 72 h inhibited the shoot height and root length of wheat seedlings by 27.7 and 41.7 %, respectively (Table 3). However, this inhibition was significantly alleviated by adding 0.5 mM SA (Table 3). Moreover, drought stress significantly decreased total fresh mass and total dry mass but SA application partially reversed this loss (Table 3).

Drought stress resulted in a dramatic accumulation of MDA in leaves of wheat seedlings (Table 4). After 72 h of drought stress, MDA content was 4.4-fold higher than before drought stress. However, this increase was 19.9 % lower when drought stress was combined with SA application.

Table 3. Effect of drought and SA application on growth characteristics of wheat seedling measured after 72 h. Means ± SD, $n = 3$. Different letters indicate a significant difference at $P < 0.05$.

Treatment	Plant height [cm]	[%]	Root length [cm]	[%]	Fresh mass [g plant^{-1}]	[%]	Dry mass [g plant^{-1}]	[%]
Control	24.67 ± 0.67a	100.0	15.23 ± 0.39a	100.0	0.45 ± 0.01a	100.0	0.065 ± 0.002a	100.0
Drought	17.83 ± 0.14c	72.3	8.87 ± 0.16c	58.3	0.24 ± 0.01c	53.3	0.043 ± 0.001c	66.2
SA + drought	20.48 ± 0.46b	83.0	11.09 ± 0.64b	72.8	0.38 ± 0.00b	84.4	0.051 ± 0.001bc	78.5

Table 4. Effect of exogenous SA on content of MDA [nmol g^{-1}(f.m.)], ASA [mmol g^{-1}(f.m.)], and GSH [mmol g^{-1}(f.m.)] in leaves of drought-stressed (0 - 72 h) wheat seedlings. Means ± SD, $n = 3$. Different letters indicate a significant difference at $P < 0.05$. There are no significant differences in MDA, ASA, and GSH content between 0 and 12, 24, 48, and 72 h in leaves of control plants, so the respective data are not shown in this table and also in Table 5.

Parameter	Treatment	0 h	12 h	[%]	24 h	[%]	48 h	[%]	72 h	[%]
MDA	drought	0.042 ± 0.004	0.064 ± 0.006a	100.0	0.077 ± 0.006a	100.0	0.120 ± 0.004a	100.0	0.186 ± 0.006a	100.0
	SA+drought		0.051 ± 0.003b	79.7	0.066 ± 0.004b	85.7	0.093 ± 0.003b	77.5	0.149 ± 0.002b	80.1
ASA	drought	0.015 ± 0.003	0.013 ± 0.001b	100.0	0.012 ± 0.002b	100.0	0.008 ± 0.001b	100.0	0.012 ± 0.003b	100.0
	SA+drought		0.015 ± 0.002a	115.4	0.013 ± 0.003a	108.3	0.016 ± 0.003a	200.0	0.018 ± 0.005a	150.0
GSH	drought	0.005 ± 0.001	0.009 ± 0.001b	100.0	0.019 ± 0.002b	100.0	0.020 ± 0.007b	100.0	0.021 ± 0.003b	100.0
	SA+drought		0.018 ± 0.004a	200.0	0.023 ± 0.004a	121.1	0.024 ± 0.008a	120.0	0.035 ± 0.004a	166.7

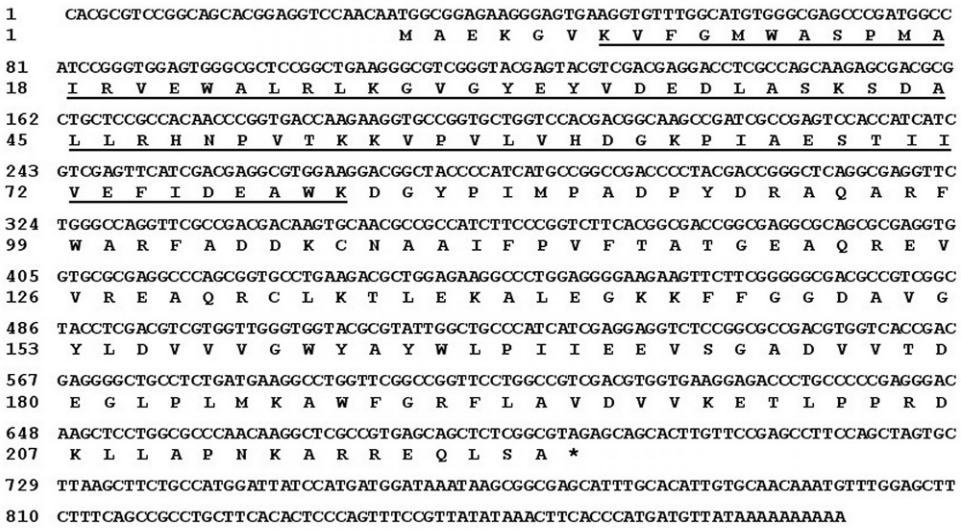

Fig. 1. The cDNA and deduced amino acid sequences of a *TaGST1* gene and its domain characterizations. GST_N_Tau domain is underlined.

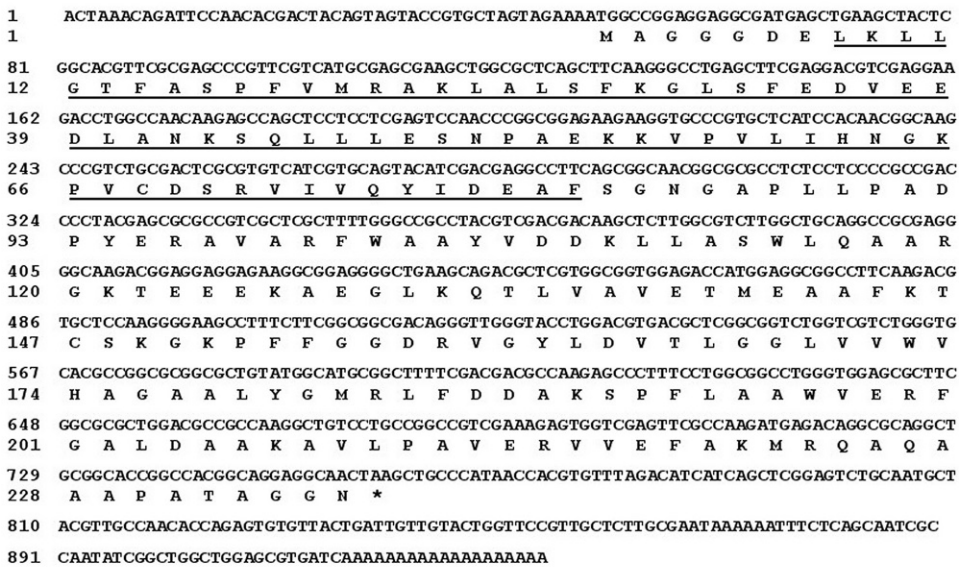

Fig. 2. The cDNA and deduced amino acid sequences of a *TaGST2* gene and its domain characterizations. GST_N_Tau domain is underlined.

Table 5. Effects of exogenous SA on the transcription of eight ASA-GSH biosynthesis-related genes under drought stress. Transcription at different time points (12, 24, 48, and 72 h) was calculated as relative to transcript level of control at 0 h = 1.00. Means ± SD, $n = 3$. Different letters indicate a significant difference at $P < 0.05$.

Gene	Treatment	0 h	12 h	[%]	24 h	[%]	48 h	[%]	72 h	[%]
GST1	drought	1.00	0.17 ± 0.01b	100.0	0.48 ± 0.03b	100.0	0.69 ± 0.06 b	100.0	1.17 ± 0.05b	100.0
	SA+drought		1.42 ± 0.01a	835.3	0.79 ± 0.08a	164.6	1.26 ± 0.05 a	182.6	2.45 ± 0.01a	209.4
GST2	drought	1.00	0.16 ± 0.00b	100.0	2.08 ± 0.20b	100.0	3.75 ± 0.14 b	100.0	4.25 ± 0.34b	100.0
	SA+drought		1.27 ± 0.04a	793.6	2.63 ± 0.20a	126.4	4.82 ± 0.34 a	128.5	6.18 ± 0.15a	145.4
GPX1	drought	1.00	0.69 ± 0.05a	100.0	0.59 ± 0.02a	100.0	0.73 ± 0.08 b	100.0	2.11 ± 0.16a	100.0
	SA+drought		0.48 ± 0.02b	69.6	0.62 ± 0.03a	105.1	1.10 ± 0.02 a	150.6	1.49 ± 0.08b	70.6
GPX2	drought	1.00	1.65 ± 0.02b	100.0	1.87 ± 0.03b	100.0	1.38 ± 0.05 a	100.0	0.75 ± 0.04a	100.0
	SA+drought		1.92 ± 0.09a	116.4	2.11 ± 0.11a	112.8	1.19 ± 0.04 b	86.2	0.37 ± 0.01b	49.3
GR	drought	1.00	0.68 ± 0.08b	100.0	9.58 ± 0.10b	100.0	10.57 ± 0.43 b	100.0	12.56 ± 0.56b	100.0
	SA+drought		9.17 ± 0.67a	1349.1	10.79 ± 0.47a	112.6	13.28 ± 0.78 a	125.6	16.83 ± 0.34a	134.0
DHAR	drought	1.00	2.19 ± 0.08b	100.0	3.80 ± 0.11a	100.0	2.82 ± 0.25 a	100.0	1.81 ± 0.31a	100.0
	SA+drought		2.69 ± 0.08a	122.8	2.87 ± 0.15b	75.5	1.68 ± 0.19 b	59.6	0.42 ± 0.05b	23.2
MDHAR	drought	1.00	0.72 ± 0.04b	100.0	1.75 ± 0.26b	100.0	2.32 ± 0.41 b	100.0	4.28 ± 0.11b	100.0
	SA+drought		1.79 ± 0.31a	248.6	3.45 ± 0.18a	197.1	5.50 ± 0.52 a	237.1	11.68 ± 0.23a	272.9
GSHS	drought	1.00	1.21 ± 0.14b	100.0	3.25 ± 0.11b	100.0	5.22 ± 0.16 b	100.0	10.36 ± 0.54a	100.0
	SA+drought		2.33 ± 0.09a	192.6	3.98 ± 0.12a	122.5	6.38 ± 0.29 a	122.2	7.86 ± 0.29b	75.9

ASA content in leaves decreased quickly with prolonged drought stress but SA application inhibited this effect (Table 4). After 72 h of drought stress, ASA content in the SA + drought treated plants was 50 % higher than in those only exposed to drought. In contrast, GSH content increased gradually during drought stress up to 4.3-fold after 72 h. Exogenous SA further accelerated this increase in GSH under drought conditions (Table 4).

The isolated full-length cDNA sequences of the *TaGST1* and *TaGST2* genes were 880 and 935 bp, respectively (GenBank acc. No. JX051003 and JX051004). They contained 666 and 711 bp ORFs flanked by 29 and 47 bp 5′-untranslated regions (UTR) and 195 and 177 bp 3′-UTR with polyA tail, respectively (Figs. 1, 2). The deduced amino acid sequences of both *TaGST1* and *TaGST2* genes contained GST_N_Tau domains (Figs. 1, 2) and showed relatively high similarities to those of *GST1* and *GST2* in other higher plants, respectively (data not shown).

The transcription of the *GST1*, *GST2*, *GPX1*, *GR*, *MDHAR*, and *GSHS* genes steeply decreased at first 12 h of drought stress. Then, their expression patterns were different to some extent. *GPX1* expression was almost stable at 24 and 48 h followed by a quick increase at 72 h, *GST1* and *MDHAR* gradually increased whereas *GST2*, *GR*, and *GSHS* quickly increased. The *GPX2* and *DHAR* gene expressions quickly increased, peaked at 24 h of drought stress, and then gradually decreased (Table 5). During almost the entire drought period, exogenous SA enhanced the transcript levels of the *GST1*, *GST2*, *GR*, and *MDHAR*. In contrast, SA application significantly increased the transcript levels of the *DHAR* gene only at 12 h, the *GPX1* gene only at 48 h, the *GPX2* gene at 12 h and 24 h, and *GSHS* at 12 h, 24 h, and 48 h (Table 5).

Discussion

In the present study, the presence of exogenous 0.5 mM SA decreased the growth inhibition caused by drought stress as manifested by less decreased plant height, root length, total fresh mass, and total dry mass (Table 3). This suggests that exogenous SA increases the tolerance of wheat seedlings to drought stress similarly to the previous reports showing that pre-treatment with SA alleviated the growth inhibition caused by drought stress in barley, bean, and tomato plants (Senaratna *et al*. 2000, EI-Tayeb 2005).

Abiotic stressors such as drought and high temperature lead to oxidative stress by increasing ROS production (Thapa *et al*. 2011). Application of SA improves the activities of antioxidative enzymes and decreases lipid peroxidation in many higher plants suffering from abiotic stresses (reviewed by Horváth *et al*. 2007b, Ashraf *et al*. 2010). These studies suggest that SA may act as a potential scavenger of ROS generated under the stress. Our results indicate that drought significantly increased lipid peroxidation (as determined by increased MDA content), but this was significantly alleviated by the addition of SA (Table 4) suggesting that SA reduces lipid peroxidation induced by drought stress.

Under stress conditions, ASA and GSH, two important members of the plant antioxidant system, destroy ROS in chloroplasts (Kuźniak and Skłodowska 2001, Ma *et al*. 2008, Bai *et al*. 2009). In two previous studies, SA pretreatment significantly accelerated the accumulation of GSH and ASA in eggplant and banana subjected to cold

stress (Kang *et al*. 2003, Chen *et al*. 2011). Our results also show that application of SA significantly increases the content of GSH and ASA in drought-stressed wheat seedlings (Table 4) suggesting that the reduced lipid peroxidation and enhanced drought tolerance may be associated with the elevated content of GSH and ASA.

Gene expression in response to abiotic stressors provides a more precise estimation of antioxidant gene activation than does enzyme activity (Shan and Liang 2010, Chen *et al*. 2011, Liu *et al*. 2012). Therefore, the transcription of eight selected genes in leaves of wheat seedlings under drought stress conditions was determined using q-PCR in the present study (Table 5). Because the cDNA sequences of the *TaGST1* and *TaGST2* genes have not yet been reported, we firstly isolated the full-length cDNA sequences using RACE (Figs. 1, 2). Then the transcript levels of the eight ASA-GSH cycle-related genes were further explored in drought-stressed wheat seedlings. We found that during the entire drought stress period exogenous SA significantly enhanced the transcription of the *GST1*, *GST2*, *GR*, and *MDHAR* genes. The transcription of *GPX1*, *GPX2*, *DHAR*, and *GSHS* induced by SA showed time-dependent manner. *DHAR* gene at 12 h, *GPX1* gene at 48 h, *GPX2* gene at 12 and 24 h, and *GSHS* at 12, 24, and 48 h of drought stress were significantly induced by SA application, respectively (Table 5). This suggests that exogenous SA could differentially regulate the expression profiles of these genes which might induce the changes in the content of GSH and ASA under drought stress (Table 4). These results are supported by two previous reports in eggplant and maize in which the transcript levels of diverse ASA-GSH cycle-related genes temporarily responded to cold and drought, respectively (Chen *et al*. 2011, Liu *et al*. 2012).

The expression profiles of the *GST1*, *GST2*, *GPX1*, *GPX2*, *DHAR*, and *MDHAR* genes regulated by exogenous SA show two-peak curves whereas the *GR* and *GSH* genes exhibit single-peak curves in cold-stressed eggplant (Chen *et al*. 2011). Our results differed and the expression profiles of the *GST1*, *GST2*, *GPX1*, *GR*, *MDHAR*, and *GSHS* genes increased gradually in controls and SA-treated wheat seedlings under drought stress whereas *GPX2* and *DHAR* exhibited single-peak curves with a peak at 24 h of drought stress (Table 5). The results suggest that the expression profiles of these genes are differentially regulated by SA under cold and drought stress conditions. The discrepancy in the expression profiles of these genes may be partially due to differences in plant species.

In conclusion, adding 0.5 mM SA significantly alleviates the inhibition of growth, reduces lipid peroxidation, and effectively protects wheat seedlings from drought stress damage. This enhanced tolerance could be related to the increased transcription of the ASA-GSH cycle-related genes and the increased content and biosynthesis of ASA and GSH. Our results contribute to elucidation of the effect of SA on the plant stress response.

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