Expression of some stress-responsive genes in tomato plants treated with ABA and sulfonamide compounds

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Abstract

Drought causes an increase in some gene expression in plant tissues such as plasma membrane intrinsic proteins type 1 (PIP1), 9-cis-epoxycarotenoid dioxygenase (NCED) SlAREB1. The effects of exogenous abscisic acid (ABA) and two sulfonamide compounds, namely, sulfacetamide (Sa) and sulfasalazine (SS) were studied on gene expression of tomato (*Lycopersicon esculentum Mill*. Cv. Super chief) under drought stress. We extracted these three genes from *Lycopersicon esculantum Mill*. Cv. Super chief leaves treated with ABA, sulfacetamide (Sa) and sulfasalazine (SS) after 24 h and 144 h of drought conditions. All treatments caused an increase in LePIP1, LeNCED1, and SlAREB1 genes expression under drought stress, leading to maintenance of the life potential of tomato plants under water deficit. It seems that these genes help the tomato plants to live under drought conditions.

Keywords: Abscisic acid; sulfacetamide; sulfasalazine; LeNCED; LePIP1

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Introduction

Plant growth and productivity are affected by various stresses such as drought, heavy metals, extreme temperature, and salinity. The mechanism of plants' responses to different stresses have been investigated by studying the genes regulation pattern under stress conditions (Zhu, 2002). Drought stress induces the expression of various genes that are involved in stress tolerance and response.

ABA is a plant hormone that is involved in stress responses, and is quickly accumulated by many plant species when exposed to drought stress. Application of ABA promotes stomatal closure (Leung and Giraudat, 1998) and induces the expression of stress-related genes such as rab18, kin 1, and rd 29B (Kurkela and Franck, 1990; Lang and Palva, 1992). It has been proposed that ABA is synthesized from carotenoids (C_{40}) in plants (Zeevaart and Creelman, 1988).

Three genes that participate in ABA biosynthesis have been isolated. They encode zeaxanthin epoxidase (ZEP) that catalyzes the epoxidation of zeaxanthin to produce epoxycarotenoid (Marin et al., 1996), 9-cis-epoxycarotenoid dioxygenase (NCED) that catalyzes the cleavage reaction of epoxycarotenoids to produce xanthin (Schwartz et al., 1997), and abscisic aldehyde oxidase (Seo et al., 2000), that catalyzes the final step of ABA biosynthesis, which converts ABA aldehyde to

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ABA. Previous reports showed that cowpea NCED gene VuNCED1 is strongly induced by drought stress in leaves (Luchi et al., 2001). Biochemical studies indicate that a key step in ABA biosynthesis is the cleavage of 9-cis-epoxycarotenoid (Sindhu and Walton, 1988). Furthermore, expression of tomato NCED gene causes overproduction of ABA in tomato (Thompson et al., 2000), which suggests a key regulatory role of NCED in ABA biosynthesis. Overexpression of the AiNCED3 cDNA in *Arabidopsis* enhances the expression of drought-inducible genes and decreases leaf transpiration through the accumulation of ABA. This overexpression of AtNCED3 confers drought tolerance to plants (Xia et al., 2010).

Some genes such as ABF2 (ABRE- binding factor 2)/AREB1 (ABA-response element binding factor 1) (Atlg45249), ABF4/AREB2 (At3gl9290) have been identified by a yeast one-hybrid system to interact with ABREs (ABA-responsive elements, PyACGTGG) in vitro (Uno et al., 2000). Expression of AREB1, AREB2, and AREB3 genes are induced by drought, salt, and ABA treatments in vegetative tissues (Uno et al., 2000). Furthermore, ABA-induced phosphorylations of AREBs through SnRKs (SNF1-related protein kinase) are important for activation of ABA-responsive genes by AREBs (Fujii and Zhu, 2009; Furihata et al., 2006). It has been reported that AREB1 functions as a prerequisite for seedling growth regulation and glucose response, conferring tolerance to multiple stresses, including drought, salt, heat, and oxidative stresses, suggesting that AREB1 plays a vital role in adaptation to these stresses (Kim et al., 2004). Overexpression of AREB2 results in enhanced ABA and salt sensitivities, and drought tolerance, demonstrating that AREB2 plays distinct roles in ABA and stress responses (Kim et al., 2004).

Expressions of SlAREB1 and SlAREB2 are induced by drought in both leaves and root tissues, although that of SlAREB1 was more affected (Orellana et al., 2010). In this study, we report the expression of SlAREB1 in tomato leaves treated with exogenous ABA and two sulfonamide compounds (SS and Sa) under drought conditions.

Aquaporins are proteinaceous pores that function as channel proteins, selectively allowing the flow of water molecules across biological membranes (Chaumont et al., 2001). The plant aquaporins are classified into plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), small basic intrinsic proteins (SIPs), and Nod 26-like intrinsic proteins (NIPs) (Chaumont et al., 2001; Steudle, 2000). The genes encoding plant aquaporins are expressed in several plant tissues, and are regulated by certain environmental stress factors such as drought, salinity, and cold (Sakurai et al., 2005). PIP proteins are divided into PIP1 and PIP2 (Schaffner, 1998). The responses of aquaporins to water stress can lead to up- or down-regulation of gene expression or even no change depending on the duration and intensity of stress (Galmes et al., 2007).

In this study, we analyzed the expression of genes encoding LeNCED1, SlAREB1 and PIP1-1 in leaf tissues. The data obtained suggest that these genes are involved in the control of water deficit tolerance in tomato.

Materials and Methods

Plant materials and growth conditions

Chemicals, ABA, sulfacetamide, and sulfasalazine were commercially purchased (Sigma-Aldrich Chemie GmbH Riedstrasse2, Fluka). Experiments were conducted in a growth chamber with 16/8 h (light/dark) photoperiod and the thermoperiod was 25/15 ˚C. Tomato plants (*Lycopersicon esculentum* Mill. cv. Super chief) were grown in pots (15 × 20 cm) containing sandy soil (5:1). Pots were continuously watered until seeds were germinated. After germination, the pots were watered adequately with one-fourth powered Hoagland nutrient solution.

Eight-week plants were exposed to drought stress by water withholding for 7 days. During drought treatment, eight groups of spraying on foliage plants were designed: 1) 10 mg/l ABA, 2) 25 mg/l ABA, 3) 25 mg/l Sa (sulfacetamide), 4) 50 mg/l Sa, 5) 100 mg/l Sa, 6) 25 mg/l SS (sulfasalazine), 7) 50 mg/l SS, and 8) 100 mg/l SS. The 9th group was considered as the control, without any application of solutions. Samplings were obtained 24 hours and 144 hours after treatments. There were three replicates for each treatment.

Table 1
Primer sequences used for LePIP1-1, LeNCED1, SlAREB1, and LeEF1 (as reference gene)

Genes	Forward Primer (5'→3')	Reverse Primer (5'→3')
LePIP1-1	AGGATCTGAATTCAATCATTT	CGGAAAAGGCAAGGTACT
LeNCED1	CTTATTTGGCTATCGCTGAACC	CCTCCAACTTCAAACTCATTGC
S1AREB1	CAGGTTTAATGGCTGGTAGTATCCC	CCATGGACCAGTTTGTGTCCGTCTTAAGC
LeEF1	TGGCCCTACTGGTTTGACAACTG	CACAGTTCACTTCCCCTTCTTCTG

RNA extraction and RT-PCR

The experiment was done according to the method described in Kim et al. (2004) with slight modifications. Total RNA was isolated from leaves with the Trizol reagent (sigma-Aldrich, USA). Dnase-I (Fermentase) treated RNA was reverse-transcribed using a high capacity cDNA reverse transcription Kit (Fermentase) and cDNA was used for RT-PCR. The PCR reaction was performed with pre-incubation at 95 ˚C for 4 min and followed by 30 cycles of denaturation consisting of 15 s at 95 ˚C and 45 s at 57 ˚C for LeNCED1, 30 s at 94 ˚C, 30 s at 40 ˚C and 90 s at 72 ˚C for PIP1-1, 30 s at 94 ˚C, 30 s at 58 ˚C and 45 s at 72 ˚C for SlAREB1, and 30 s at 95 ˚C, 45 s at 50 ˚C and 45 s at 72 ˚C for LeEF1. The LeEF1 gene (Elongation Factor 1) was used as internal reference. Forward and reverse primers sequences used for LePIP1-1, LeNCED1, SlAREB1 and LeEF1 are listed in table 1. Experiments repeated three times. The intensity of the RT-PCR bands was measured using Image J software 1.43.

Statistical Analysis

Statistical analyses were done using SPSS. Error bars on graphs are standard error of mean. One-way analysis of variance with posttests with Tukey's multiple range tests ($p<0.05$) was used to determine differences between means.

Results

During drought period, the expression level of genes LeNCED1, LePIP1-1, and SlAREB1 changed (Fig. I). In plants sprayed with ABA 10 mg/l, SS (25 and 50 mg/l), and Sa 100 mg/l, the expression level of LeNCED1 increased during stress time. No significant changes in gene expression of LeNCED1 was observed in leaves sprayed with ABA 25 mg/l but a gene expression of LeNCED1 was observed in leaves sprayed with SS 100 mg/l and Sa 50 mg/l (Fig. II). The difference between SS, Sa, and control plants was significant ($p<0.05$).

After 144 h of stress period, the gene expression of LePIP1-1 increased in leaves

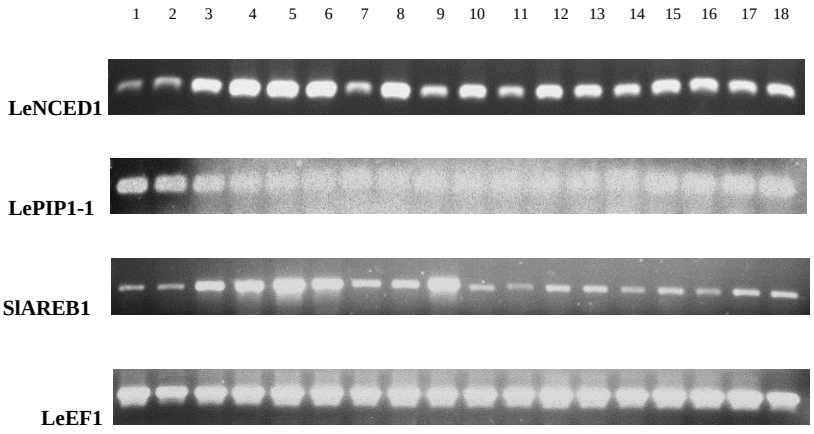

Fig. I. Expression profiles of *LeEF1* in tomato leaves under drought stress; Samples of 1-9 were gathered 24 h after stress period and samples of 10-18 were gathered 144 h after drought time: 1,10) control; 2,11) ABA 10; 3,12) ABA 25; 4,13) SS 25; 5,14) SS 50; 6,15) SS 100; 7,16) Sa 25; 8,17) Sa 50; 9,18) Sa 100 (ABA: Abscisic acid, Sa: Sulfacetamide, SS: sulfasalazine)

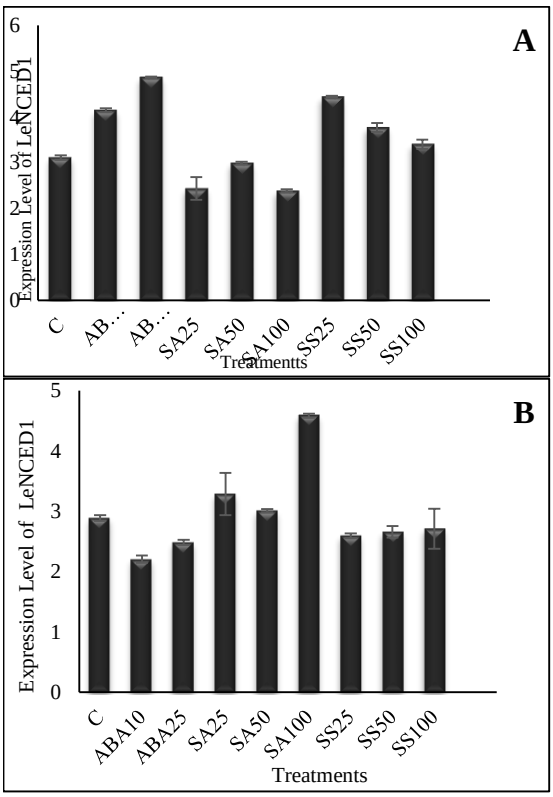

Fig. II. The expression level of LeNCED1 after 24 hours (A) and 144 hours (B) in tomato leaves sprayed with ABA, SS and Sa; C: Control, ABA: Abscisic acid, Sa: Sulfacetamide, SS: sulfasalazine

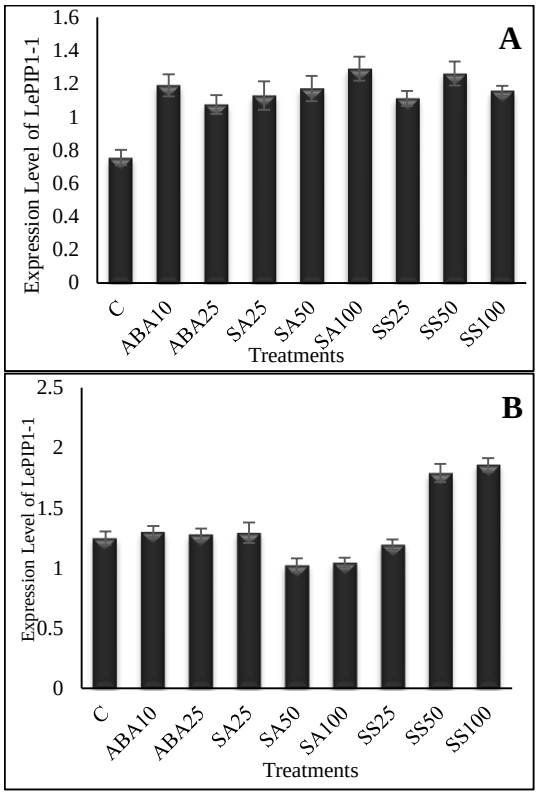

Fig. III. The expression level of LePIP1-1 after 24 hours (A) and 144 hours (B) in tomato leaves sprayed with ABA, SS and Sa; C: Control, ABA: Abscisic acid, Sa: Sulfacetamide, SS: sulfasalazine

recipient of the ABA (10 and 25 mg/l), SS 25 mg/l, and Sa (50 and 100 mg/l) solutions. In plants sprayed with SS 50 mg/l solution, the expression of LePIP1-1 showed no significant change (Fig. III). Therefore, it can be concluded that LePIP1-1 expression increased dramatically in leaves sprayed with Sa (50 and 100 mg/l) and SS 25 mg/l solutions. The gene expression of SlAREB1 showed a significant increase ($p<0.05$) in leaves sprayed with ABA 10 mg/l, SS 50 mg/l, and Sa 25 mg/l solutions, and it decreased in plants receiving SS 25 and 100 mg/l solutions. SlAREB1 expression had no significant changes in leaves sprayed with ABA 25 mg/l and Sa 25 mg/l solutions (Fig. IV).

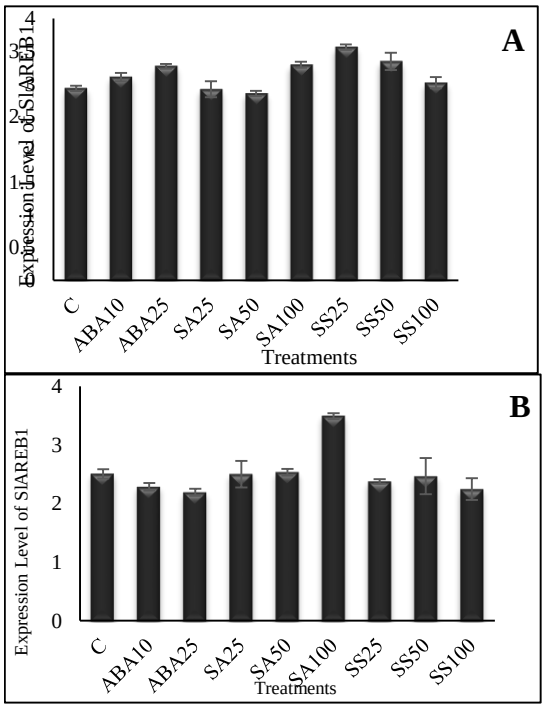

Fig. IV. The expression level of SlAREB1 after 24 hours (A) and 144 hours (B) in tomato leaves sprayed with ABA, SS and Sa; C: Control, ABA: Abscisic acid, Sa: Sulfacetamide, SS: sulfasalazine

Discussion

Drought is one of the most common stressful conditions that plants experience in their environment, and most frequently it has a negative effect on hydraulic conductance of the roots (Vandeleur et al., 2005). Under transpiring conditions water comes from the soil to the root xylem following mainly the apoplastic path

governed by a hydrostatic pressure gradient; however, when transpiration is restricted by stressful conditions such as drought, more of the water follows the cell-to-cell path, flowing across membranes of living cells (Steudle, 2000).

The 9-cis-epoxycarotenoid cleavage reaction is thought to be a rate-limiting step in ABA biosynthesis. This reaction is catalyzed by NCED genes that are often encoded by a small gene family. Previous studies provided evidence that LeNCED1 is positioned in the upper part of the ABA pathway (Xia et al., 2003). To examine the role of NCED in ABA biosynthesis under drought stress condition in the tomato leaves treated with exogenous ABA, SS, and Sa, we isolated NCED genes (LeNCED) from tomato genome and identified a drought-inducible NCED gene, LeNCED1. The expression of LeNCED1 was strongly induced by drought stress and the applied solutions (Fig. II), thus LeNCED1 should be the major NCED gene in tomato involved in the regulation of ABA levels under drought conditions as this was reported previously (Xia et al., 2003). Luchi et al. (2001) have reported that AtNCED genes (except for AtNCED9) are strongly induced by drought stress. It has been reported that the expression of AtNCED1 was induced by drought stress in *Arabidopsis thaliana* ecotype landsberg (Neil et al., 1998). Drought inducible NCED genes have been also reported in maize (Schwartz et al., 1997), tomato (Burdbidge et al., 1997), bean (Qin and Zeevaart, 1999), cowpea (Luchi et al., 2000), and avocado (Chernys and Zeevaart). As NCED is thought to function in the rate-limiting step of ABA biosynthesis, drought-inducible homologues for NCED may be responsible for the accumulation of ABA under drought-stressed conditions. It is noteworthy that overexpression of LeNCE1, a tomato NCED, elevates endogenous ABA in transgenic tobacco leaves (Thompson et al., 2000). In the present study we showed that the expression of LeNCED1 in tomato plants treated with ABA, SS, and Sa was induced in drought conditions (Fig. II).

In drought condition, regulation of water status in plants is highly important and complex. Aquaporins activity may be one way of maintaining an appropriate water balance when water availability becomes a growth-limiting factor (Schaffner, 2005). Here, mRNA expression of the LePIP1-1 gene increased in leaf tissues as a result of drought (144 h after water withholding) (Fig. III). *Arabidopsis thaliana* has five PIP1 and eight PIP2 proteins (Johonson et al., 2003), whereas *Zea mays* has six PIP1 and seven PIP2 protein (Chaomont et al., 2001); and, three PIP1 and eight PIP2 proteins were found in rice (Sakarai et al., 2005). ABA, SS and Sa treatment raised the expression of PIP1-1 gene in leaves of tomato plants (Fig. III). Aroca et al. (2006) have reported that PIP1 protein in the *phaseolus vulgaris* roots rose under drought treatment. It has been reported that the expression levels of OsPIP1-1, OsPIP1-2, OsPIP2, and OsPIP2-3 in the leaf tissues of *japonica rice* cv. Zhonghua 11 exposed to 20% PEG 6000 induced water deficit were upregulated (Gao et al., 2006).

In the present study, among AREB transcription factors, SlAREB1 is described in tomato leaves. We provide evidence that the expression of SlAREB1 correlated with duration of drought conditions. AREB/ABF bZIP transcription factors have been described to mediate stress-associated gene regulation in aerial tissues and roots in *Arabidopsis* (Kang et al., 2002; Fujita et al., 2005), and rice (Amir Hossain et al., 2010). In tomato, database scrutiny has delivered only three homologous sequences here named SlAREB1, SlAREB2, and a third sequence that only covers the N-terminal half with the typical conserved regions. Also, another characteristic of these factors is that they are induced by ABA (Choi et al., 2000; Uno et al., 2000). Transcription of SlAREB1 and SlAREB2 was also shown to be upregulated by ABA. In the present study, the corresponding SlAREB1 factor from *Lycopersicum esculantum* cv. super chief was described and induced by ABA, SS and Sa (Fig. IV).

In conclusion the present study suggests that ABA, SS, and Sa treatments increase the expression of PIP1-1 gene in leaves of tomato plants. Also the expression of SlAREB1 results in a noticeably improved tolerance to water deficit compared to control plants. Our results indicate that endogenous ABA levels can be manipulated by controlling the expression levels of LeNCED genes in the treated plants.

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