Volume 55(1):83-85, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Changes in aldehyde oxidase activity and gene expression in *Solanum lycopersicum* L. shoots under salicylic acid pre-treatment and subsequent salt stress

Edit Horváth*, Ágnes Gallé, Ágnes Szepesi, Irma Tari and Jolán Csiszár

Department of Plant Biology, University of Szeged, Szeged, Hungary

ABSTRACT The plant hormone abscisic acid (ABA) plays essential role in adaptation and response processes in plants by modifying a number of gene expressions. Aldehyde oxidases (AOs) may participate in stress responses, because it catalyzes the oxidation of abscisic aldehyde to ABA, in the last step of ABA synthesis. In this work the expression levels of the three known tomato AO genes were monitored by quantitative real-time PCR (QRT-PCR) after 3-weeks pre-treatment with two salicylic acid (SA) concentrations followed by 1-week-long salt stress. The 10^{-4} M SA pre-treatment, but not the 10^{-7} M SA, increased the *SlAO2* gene expression, and both SA concentrations increased the transcript amount of *SlAO3*. The high salinity treatment for one week increased slightly only *SlAO2* gene expression in the control shoots. The AO1 enzyme activity, which was studied with native polyacrylamide gel electrophoresis, was on control level after 4-week pre-treatment with 10^{-4} M SA, but decreased using 10^{-7} M SA. One-week 100 mM NaCl treatment decreased the activity of the AO1 isoenzyme excluding the 10^{-7} M SA pre-treatment. These moderate changes in the gene expressions and enzyme activities may contribute in the acclimation of 10^{-4} M SA pre-treated tomato plants to subsequent salt stress.
Acta Biol Szeged 55(1):83-85 (2011)

KEY WORDS

NaCl stress
aldehyde oxidase
Solanum lycopersicum
salicylic acid

Salinity is one of the major abiotic stresses, which adversely influence the crop productivity and quality (Chinnusamy et al. 2005). High salt concentrations cause ionic and osmotic stresses (Zhu 2001), and salt response processes activate the expression of a large number of genes in tomato plants already in early stages, this imply on a very complex trait of tomato salt tolerance (Ouyang et al. 2007).

The plant hormone abscisic acid (ABA) is important in adaptation to environment, responses to environmental stresses and induction of stress-related gene expression. Salt stress induces ABA accumulation through transcriptional regulation of ABA biosynthesis genes (Xiong and Zhu 2003). Aldehyde oxidases (AOs) catalyze the last step of ABA synthesis, the oxidation of abcisic aldehyde to ABA. In Arabidopsis there are evidences that from the four known AO genes the *AtAAO3* gene encodes the enzyme which catalyzes the final step of ABA biosynthesis (Seo et al. 2000). From the three known AO genes in pea only the *PsAO3* expression increased under salt stress in roots and in leaves of pea plant, moreover *PsAO3* might encode the PAO-γ isoform, which participates in stress induced ABA synthesis (Zdunek- Zastocka 2008). In tomato three AO genes (*AO1*, *AO2* and *AO3*) and two pseudogenes (*AO4* and *AO5*) were described (Ori et al. 1997; Min et al. 2000), but it is still unclear which one is involved in salt stress responses. In our earlier experiments, 10^{-7} M and 10^{-4} M SA pre-treatment of tomato plants facilitate the acclimation to subsequent salt stress induced with 100 mM NaCl. SA induced ABA accumulation in leaves and roots and also increased the aldehyde oxidase activity (Szepesi et al. 2009).

In this work we studied the relative transcript expression level of known tomato AO genes (*SlAO1*, AF258808; *SlAO2*, AF258809; *SlAO3*, AF158810) in shoots with quantitative real-time PCR (QRT-PCR) after 3 weeks pre-treatment with two SA concentrations followed by 1-week-long salt stress.

Materials and Methods

Solanum lycopersicum Mill. L. cvar. Rio Fuego was grown in a greenhouse in perlite for seven days and after that they were cultivated in hydroponic culture for two weeks. The plants were grown for 12 hours in the light and 12 hours in the dark. The light intensity and relative humidity were 180 μmol m^{-2} s^{-1} and 55-60%. Three-week-old plants were pre-treated with 10^{-7} M and 10^{-4} M SA for three weeks, and than the plants were treated with 100 mM NaCl to induce salt stress. The hydroponic medium contained SA throughout the experimental period. Samples were taken after one week salt treatment.

Accepted July 11, 2011
*Corresponding author. E-mail: horvathedo@yahoo.com

Table 1. Gene-specific primers used for QRT-PCR. Three primer pairs amplify tomato aldehyde oxidase sequences, the last two primer pairs were utilised to amplify low- and high-expression standards.

Primer	Description	Sequence 5′-3′
AF258808 F	Aldehyde oxidase 1	CCAGGCACAAACACAATCAA
AF258808 R		GTCGTAAATAATATCAGACTG
AF258809 F	Aldehyde oxidase 2	TTCCAGACGAAGACAACTGC
AF258809 R		GACAAGTGGCAATCACACGA
AF258810 F	Aldehyde oxidase 3	AGTTGGCAGTGTCCTCAAGC
AF258810 R		GACTTCATACACGATTGGCACT
X51576 F	18S rRNA	CGGAGAGGGAGCCTGAGAA
X51576 R		CCCGTGTTAGGATTGGGTAATTT
U97257 F	Glyceraldehyde 3-phosphate	ACAACTTAACGGCAAATTGACTGG
U97257 R	dehydrogenase	TTACCCTCTGATTCCTCCTTGATTG

Aldehyde oxidase was detected by native polyacrylamide gel electrophoresis (native-PAGE) using 2- naphtaldehyde as a substrate according to Sagi et al. (1998).

Tri-reagent method was used to isolate total RNA from plant material (Chomczynski and Sacchi 1987). cDNA was prepared from 1 μg DNase-treated RNA. The expression rate was monitored with SYBR Green by QRT-PCR. Data was analysed using Opticon monitor software. Gene-specific primers, used for RT-PCR analyses were designed with Primer3 software (frodo.wi.mit.edu/primer3), primers are described in Table 1. 18S ribosomal RNA (18S rRNA; X51576) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; U97257) were used as reference genes. Data were calculated with $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Results and Discussion

Staining with 2-naphtaldehyde substrate after native-PAGE revealed that 4-week-long 10^{-7} M SA treatment caused decrease in the activity of AO1 isoenzyme in tomato shoot tissues, while it was on control level in 10^{-4} M SA treated plants. After 1-week 100 mM NaCl treatment, excluding the 10^{-7} M SA pre-treated plants, the activity of the AO1 isoenzyme decreased. Some induction was detected in activity of other isoenzymes due to SA pre-treatment.

Three tomato AO genes were identified in DDBJ/EMBL/GeneBank database and their expression levels were investigated in shoots using QRT-PCR. While the expression level of *SlAO1* did not altered, the relative mRNA amounts of *SlAO2* and *SlAO3* genes were higher in the SA pre-adapted plants compared to control, which may responsible for the elevated ABA content of plants published earlier (Szepesi et al. 2009). The 100 mM NaCl increased (*SlAO2*) or did not affect (*SlAO1*, *SlAO3*) on the expression of AO coding sequences in tomato shoots. On the contrary, after applying of 100 mM NaCl on 10^{-7} M SA or 10^{-4} M SA pre-treated plants, the expression of AO genes remained on control level.

In conclusion, our results indicate that the effect of SA on *SlAO2* and *SlAO3* gene expression can take part in the ABA accumulation observed earlier at the end of pre-treatment period. This enhanced expression can insure the proper enzyme amount for ABA biosynthesis after addition of 100 mM NaCl. This process contributes in the successful acclimation of SA pre-treated tomato plants to subsequent salt stress.

Acknowledgements

This work was supported by the OTKA K76854 grant. A part of this study was presented on the 10th Congress of the Hungarian Society for Plant Biology, August 31 - September 2, 2011, Szeged, Hungary.

References

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol– chloroform extraction. Anal Biochem 162:156-159.

Chinnusamy V, Jagendorf A and Zhu J-K (2005) Understanding and improving salt tolerance in plants. Crop Sci 45:437-448.

Horváth E, Szalai G and Janda T (2007) Induction of abiotic stress by salicylic acid signaling. J Plant Growth Regul 26:290-300.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 25:402–408.

Min X, Okada K, Brockman B, Koshiba T, Kamiya Y (2000) Molecular cloning and expression patterns of three putative functional aldehyde oxidase genes and isolation of two aldehyde oxidase pseudogenes in tomato. Biochim Biophys Acta 1493:337-341.

Ori N, Eshed Y, Pinto P, Paran I, Zamir D, Fluhr R (1997) TAO1, a Representative of the Molybdenum Cofactor Containing Hydroxylases from Tomato. JBC 272:1019-1025.

Ouyang B, Yang T, Li H, Zhang Y, Zhang J, Fei Z and Ye Z (2007) Identification of early salt stress response genes in tomato root by suppression subtractive hybridization and microarray analysis. J Exp Bot 58:507-520.

Sagi M, Omarov RT, Lips HS (1998) The Mo-hydrolases xanthine and aldehyde oxidase in ryegrass as affected by nitrogen and salinity. Plant Sci 135:125-135.

Seo M, Peeters A J M, Koiwai H, Oritani T, Mation-Poll A, Zeevaart J A D, Koorneef M, Kamiya Y and Koshiba T (2000) The Arabidopsis aldehyde oxidase 3 (AAO3) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. PNAS 97:12908-12913.

Szepesi Á, Csiszár J, Gémes K, Horváth E, Horváth F, Simon LM, Tari I (2009) Salicylic acid improves the acclimation to salt stress by stimulating abscisic aldehyde oxidase activity and abscisic acid accumulation, and increases Na+ contents of the leaves without toxicity symptoms in

Solanum lycopersicum L. J Plant Phys 166:914-925.
Szepesi Á, Poór P, Gémes K, Horváth E, Tari I (2008) Influence of exogenous salicylic acid on antioxidant enzyme activities in the roots of salt stressed tomato plants. Acta Biol Szeged 52:199-201.
Zhu J-K (2001) Plant salt tolerance. Trends Plant Sci 6:66-71.
Zdunek- Zastocka E (2008) Molecular cloning, characterization and expression analysis of three aldehyde oxidase genes from *Pisum sativum* L. Plant Phys Biochem 46:19-28.
Xiong L and Zhu J-K (2003) Regulation of abscisic acid biosynthesis. Plant Physiol 133:29-36.