Journal of Medicinal Plants Research Vol. 5(24), pp. 5889-5893, 30 October, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Meta-analysis of *Arabidopsis thaliana* under abscisic acid and salt stress

Guowu Yu[1], Weidong Zang[2#], Xianquan Yang[1], Lishan Wang[2,3#], Zongxiang Tang[4] and Peigao Luo[4*#]

[1]Department of Biotechnology, Agronomy College, Sichuan Agriculture University, 211 Huimin Road. Wenjiang District, Chengdu, Sichuan, 611130, China.
[2]Shanghai Fenghe info-Tech Co., Ltd., Shanghai, 200241, China.
[3]Department of Biology, Shanghai Jiaotong University, Dongchuan Road, Shanghai, 200241, China.
[4]State Key Laboratory of Plant Breeding and Genetics, Sichuan Agricultural University, 211 Huimin Road, Wenjiang District, Chengdu, 611130, China.

Accepted 11 August, 2011

To investigate the abscisic acid and salt stress on Arabidopsis and find the potential response genes, we collected the gene expression profiles of Arabidopsis under abscisic acid (ABA) and salt stress from NCBI, and merge the 2 different datasets by meta-analysis method. Thus, the different expressed genes identified from the merged data presented the responses of Arabidopsis under both abscisic acid and salt stress. We found that the genes ALDH7B4, ATTSPO, ANAC019, AZF2, and ATADH1 are induced upon both abiotic stress and abscisic acid and identified that expansion domain is highly related to both abiotic stress and abscisic acid.

Key words: Meta-analysis, salt stress, abscisic acid (ABA) stress, Arabidopsis.

INTRODUCTION

Abiotic stresses are believed to adversely affect agriculture by reducing crop growth and productivity. Because of their sessile nature, plants must endure adverse environmental conditions and consequently evolve a variety of responses to acclimatize to environmental stresses (Gao et al., 2007). Tolerance or susceptibility to these abiotic stresses is a very complex phenomenon, in part, because stress may occur at multiple stages of plant development and often more than one stress simultaneously affects the plant (Chinnusamy et al., 2004). During evolution, plants have developed sophisticated mechanisms to perceive the subtle changes of growth conditions, and trigger signal transduction cascades, which in turn activate stress-responsive genes and ultimately lead to changes at the physiological and biochemical levels (Xiong et al., 2001; Hazen et al., 2003). Salt stress is one of the most common abiotic stresses namely, ABA stress. And ABA-mediated signaling is suggested as one of the transduction pathways that that plant encounters. It have been identified that salinity stress would cause abscisic acid (ABA) accumulation, regulates the salt stress-responsive genes. For example, the expression of AtCBG are induced by ABA and salt stresses, and specific knock-out mutants are highly sensitive to ABA and salt treatments. These findings suggest that this protein is a ABA- and salt stress-related signal transducer (Jayasekaran et al., 2006). Similarly, the expressions of the OsMCSU gene are up-regulated by salt stress in root tissues and could be mediated by both ABA-dependent and ABA-independent signaling pathways under salt stress condition (Huang et al., 2009). In general, there is extent relationship between salt and ABA response in plant and it is immense valuable for identifying more related genes for improving salt and ABA stress in molecular technology. Meta-analysis provides a powerful tool for analyzing microarray experiments by combining data from multiple studies, and it presents unique

*Corresponding author. E-mail: luopeigao@gmail.com.

#These authors contributed to the work equally.

computational challenges. The Bioconductor package Rank Prod provides a new and intuitive tool in detecting differentially expressed genes (DEGs) under different experimental conditions (Hong et al., 2006). In this work, we measured the genome wide gene transcription profiles of Arabidopsis under ABA and salt stress. With meta-analysis method, we integrated the expression data of Arabidopsis under ABA and salt stress and identified the differently expressed genes. Other analysis methods, such as gene ontology (GO), domain enrichment analysis, were used to explained the complexity of Arabidopsis response to abiotic stress.

METHODS

Microarray analysis

Microarray data have been deposited in Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo/), with accession number GSE7112 and GSE18217. Total 8 microarrays were obtained. Each dataset uses 2 treatment and 2 controls. For the GSE7112 and GSE18217 datasets, the limma method (Smyth, 2004) was used to identify DEGs. The original expression datasets from all conditions were processed into expression estimates using the RMA method with the default settings implemented in Bioconductor, and then construct the linear model. The DEGs only with the fold change > 1.5 and p-value < 0.05 were selected. The Rank Product package (Hong and Breitling, 2006) was used to identify the DEGs between controls and treatment in each experiment. Briefly, genes were ranked based on up- or down-regulation by the treatment in each experiment. Then, for each gene a combined probability was calculated as a rank product (RP). The RP values were used to rank the genes based on how likely it was to observe them by chance at that particular position on the list of DEGs. The RP can be interpreted as a p-value. To determine significance levels, the RP method uses a permutation-based estimation procedure to transform the p-value into an e-value that addresses the multiple testing problems derived from testing many genes simultaneously. Genes with a percentage of false-positives (PFP) ≤0.05 were considered differentially expressed between treatments and control in each experiment. This method has the advantage to identify genes with a response to the Arabidopsis abiotic stress. Because we used two different sample sets from different experiment, especially the GSE7112 dataset with less DEGs. Moreover, RP has proved to be a robust method for comparing microarray data from different sources and experiments (Hong et al., 2008; Vinuela et al., 2010).

GO enrichment and IntroPro domain analysis

The Gene Ontology project, which is used to annotate and analyze the function of large numbers of genes, is a major bioinformatics initiative with the aim of standardizing the representation of gene and gene product attributes across species and databases. InterPro (Hunter et al., 2009) is a new integrated documentation resource for protein families, domains and functional sites. Gene ontology (GO) and IntroPro domain data were extracted using the DAVID (Huang da et al., 2009). GO terms and IntroPro domains with less than 2 genes were discarded. Over-represented groups of GO terms were identified using a hypergeometric test, with a threshold of p-value < 0.01. Using the same test, IntroPro domains analysis with p-value <0.05 is to find the significant domains.

RESULTS

Effects of abiotic stress on transcript levels in Arabidopsis

To get DEGs of Arabidopsis exposed to abiotic stress, we obtained publicly available microarray data sets GSE7112 and GSE18217 from GEO. After microarray analysis, the DEGs with the fold change > 1.5 and p-value < 0.05 were selected. 384 genes from GSE7112 and 5931 genes from GSE18217 were selected as DEGs. Use the RankProd packages for meta-analysis, 257 up-regulated genes and 103 down-regulated genes with a percentage of false-positives (PFP) <0.05 were considered differentially expressed. At last, 74 common genes were collected from above three datasets. Figure 1 displayed the expression values of common genes in our datasets. Each column strands for the gene and each row strands for each sample. The expression values were shown in Figure 1.

GO enrichment analysis

To gain insight into the biological processes associated with the regulated genes, we determined which GO annotation terms were over-represented. In both treatments, significantly enriched GO terms (p-value<0.01 using hypergeometric test) were related with response to water, response to osmotic stress, response to cold and response to temperature stimulus (Figure 2 and Table 1). Table 1 lists the overlap of GO enrichment analysis results enriched in the DEGs of ABA, salt and the two abiotic stresses meta-analysis.

Domain analysis

To add meaningful information to the results from the GO term enrichment we extended our investigation by using a similar analysis with protein domains associated to the regulated genes as categories. Only functional domains common and significant (p-value <0.05, hypergeometric test) within the abiotic stress are shown (Table 2). Most of significantly overrepresented groups included domains were related with expansion and pollen allergens.

DISCUSSION

We present an analysis and comparison of whole genome transcription analysis of abiotic stress. ALDH7B4, ATTSPO, ANAC019, AZF2 and ATADH1 genes were proved to be highly related to ABA and salt stress. And we also found expansin domain in response to ABA and salt stress. ALDH7B4 encodes a turgor-responsive ALDH and belongs to family 7 ALDH proteins. It plays a major role in the detoxification processes of

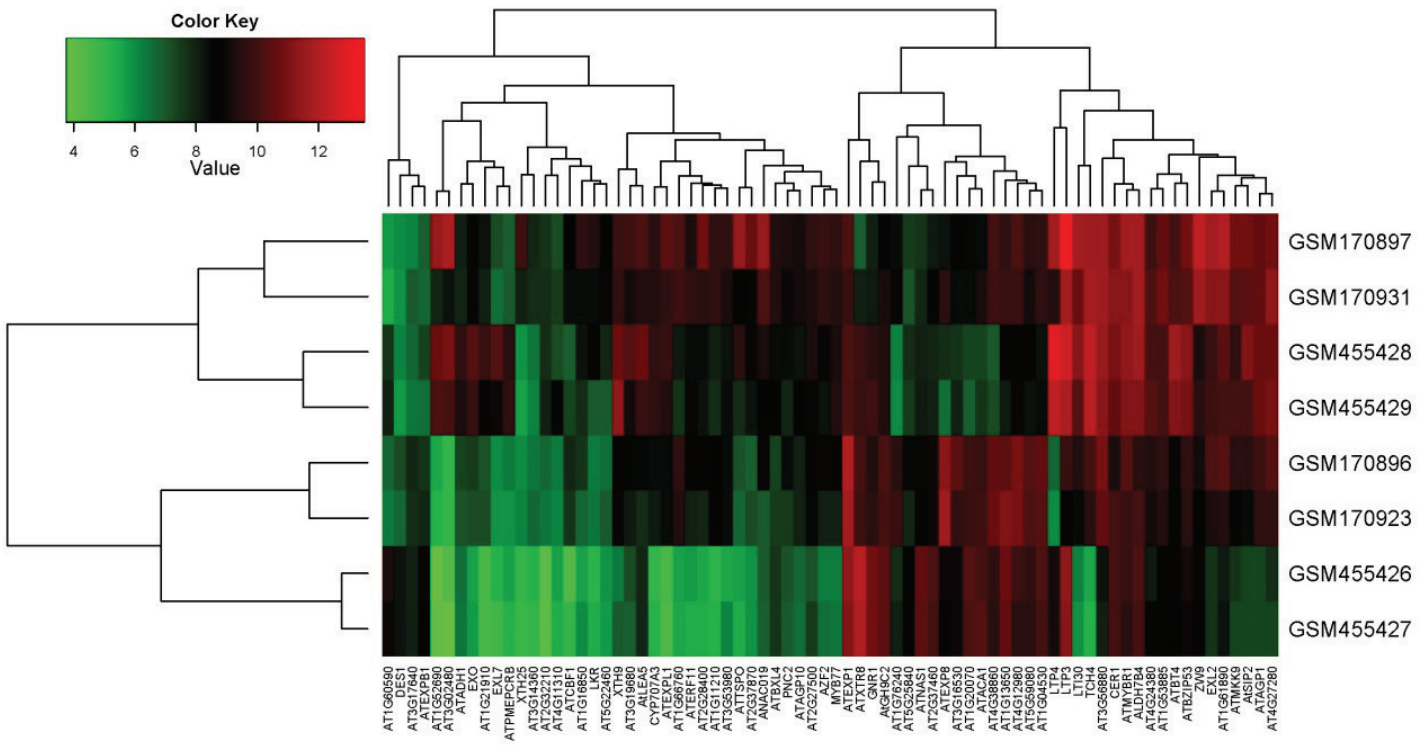

Figure 1. Expression profiles of the 74 Arabidopsis common genes under ABA and salt stress.

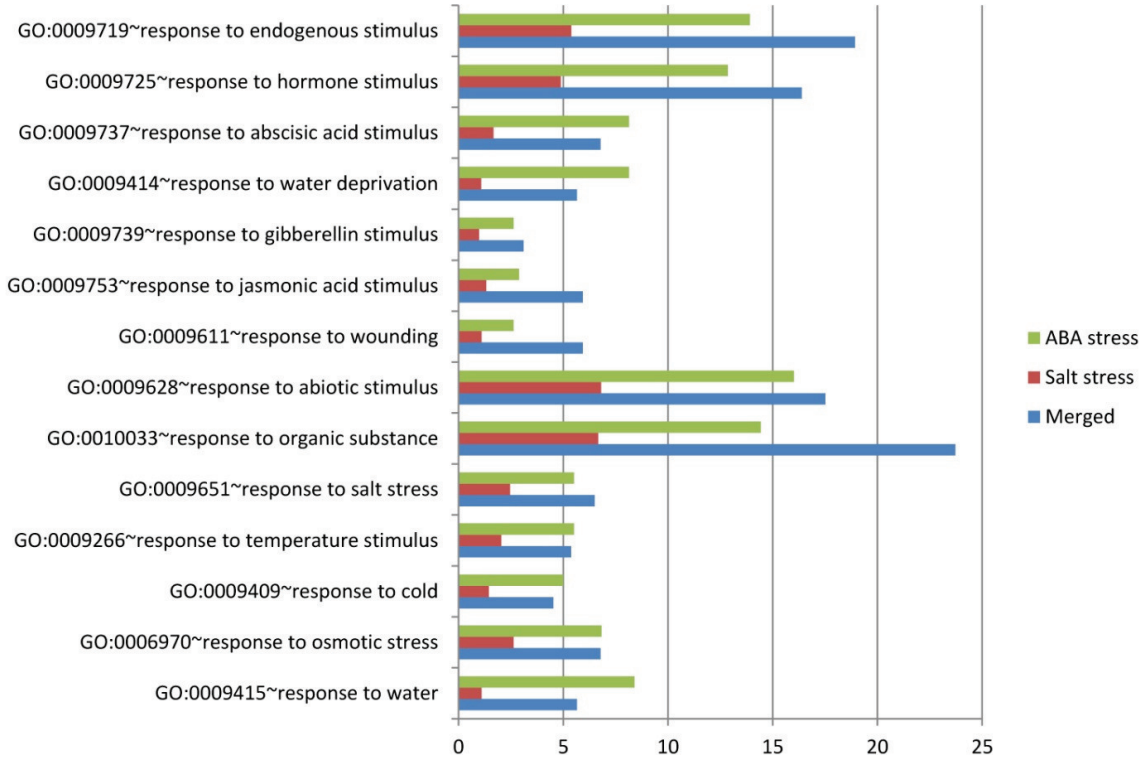

Figure 2. DEGs within enriched GO terms.Percentage of genes within each GO terms significantly regulated in each dataset: GSE7112 (green), GSE18217 (red) and a combination of both (blue). Only significantly enriched GO terms for all the treatments are shown (p-value <0.01 using hypergeometric test).

Table 1. Major gene ontology (GO) terms represented in the different samples.

Term	Merged (P-value)	Salt stress (P-value)	ABA stress (P-value)
GO:0009415~response to water	3.35E-10	1.08 E-3	9.30E-22
GO:0006970~response to osmotic stress	3.06E-07	9.72E-11	4.06E-08
GO:0009409~response to cold	1.03E-05	3.32E-05	1.66E-07
GO:0009266~response to temperature stimulus	3.39E-05	2.95E-05	4.80E-06
GO:0009651~response to salt stress	3.16E-07	1.79E-10	8.88E-06
GO:0010033~response to organic substance	2.66E-31	1.42E-13	4.19E-11
GO:0009628~response to abiotic stimulus	1.91E-15	4.77E-14	5.59E-14
GO:0009611~response to wounding	5.89E-13	2.79E-07	1.19E-03
GO:0009753~response to jasmonic acid stimulus	1.83E-12	1.10E-11	4.50E-04
GO:0009739~response to gibberellin stimulus	3.39E-05	2.65E-08	2.57E-04
GO:0009414~response to water deprivation	1.41E-10	4.37E-04	2.63E-21
GO:0009737~response to abscisic acid stimulus	5.63E-10	1.02E-04	2.84E-15
GO:0009725~response to hormone stimulus	1.53E-18	1.56E-07	5.09E-12
GO:0009719~response to endogenous stimulus	2.11E-23	1.44E-09	3.67E-13

Table 2. DEGs within enriched domains under ABA and salt stress.

Term	Meta (P-value)	Salt stress (P-value)	ABA stress (P-value)
IPR007112:Expansin 45, endoglucanase-like	1.51E-3	2.43E-2	1.65E-6
IPR005132:Rare lipoprotein A	1.51E-3	2.43E-2	1.65E-6
IPR014734:Pollen allergen, N-terminal	1.51E-3	2.43E-2	1.65E-6
IPR007117:Pollen allergen/expansin, C-terminal	1.22E-3	3.38E-2	1.09E-6
IPR007118:Expansin/Lol pI	1.22E-3	3.38E-2	1.09E-6
IPR016455:Xyloglucan endotransglucosylase/hydrolase	4.55E-6	4.71E-2	1.77E-3

aldehydes generated in plants when exposed to abiotic stress. ALDH7B4 showed a strong accumulation in plants exposed to salt stress, and ABA treatment to affect sugar metabolism and fatty acid composition of membrane lipids. Besides reducing the level of reactive aldehydes, they may supply NADH or NADPH and the corresponding carboxylic acids to the metabolism under stress conditions when the flow of redox equivalents from photosynthesis is inhibited (Kirch et al., 2005; Kotchoni et al., 2006).

ATTSPO encodes a TSPO-related membrane-bound protein. AtTSPO is mainly detected in dry seeds, but can be induced in vegetative tissues by salt stress or ABA treatment. Constitutive expression of AtTSPO resulted in increased sensitivity to NaCl. Transgenic Arabidopsis plants over-expressing AtTSPO were more sensitive to ABA-induced growth inhibition, indicating that constitutive expression of AtTSPO may enhance ABA sensitivity. Taken together, these results suggested that AtTSPO is a highly regulated protein, induced by salt stress to modulate, at least in part, transient intracellular ABA-dependent stress perception and/or signaling (Guillaumot et al., 2009; Guillaumot et al., 2009).ANAC019 encodes a NAC transcription factor. ANAC019 was identified as a new positive regulator of ABA signaling, conferring ABA hypersensitivity when ectopically expressed in plants. But, ABA plays a key role in salt-stress perception. Therefore, expression of the ANAC019 gene could be induced when in salt and ABA stresses (Jensen et al., 2010). AZF2 encoding Cys(2)/His(2)-type zinc-finger protein which belong to the group of ZPT2-related proteins in Arabidopsis. RNA gel-blot analysis showed that expression of AZF2 was strongly induced by high-salt and ABA treatment. The level of AZF2 mRNA increased slowly and peaked within 24 h with high-salt and ABA treatments. Further study indicated that the high-salt-responsive expression of AZF2 was mainly induced via an ABA-independent pathway. AZF2 functions as transcriptional repressors to increase stress tolerance following growth retardation (Sakamoto et al., 2004). AtADH1 catalyzes the reduction of acetaldehyde using NADH as reductant. AtADH1 could be induced expression in ABA treatment and salt stress at the participation of many transcription factors. Such as, over-expression of the Arabidopsis bZIP factors ABF3 and ABF4 resulted in the over-expression of AtADH1 under high-salt conditions (Kang et al., 2002). In 35S:AtMYB2, 35S: AtMYC2 and 35S: AtMYC2/AtMYB2 plants, the

expression of AtADH1was induced increasing with ABA treatment (Abe et al., 2003). In addition, the transcript levels of AtADH1 were identified generally higher in MYB15 over-expression seedlings than in WT controls after ABA treatment and NaCl stress (Ding et al., 2009).Expansins are cell wall loosening proteins that appear to permit the microfibril matrix network to slide in growing plant cell walls, thereby enabling the wall to expand. Previous salt and ABA stress studies in sorghum documented that expression of a beta-expansin gene family (BG051261) was increased >100-fold selectively in sorghum shoots in response to ABA treatment (Buchanan et al., 2005). Recent study indicated that both over-expression AtEXP3-OX and AtXPb1-OX plants exhibited increased sensitivity to salt stress, but they did not display any distinct differences to wild type in different ABA treatment (Kwon et al., 2008). In this report our analysis has focused on the genes which are significantly differentially expressed, as determined by Rank Products Analysis. Our analysis of these genes indicated expression of ALDH7B4,ATTSPO , ANAC019 , AZF2 , ATADH1 are induced upon abiotic stress and we are able to identify expansin domain is induced in response to ABA and salt stress.

ACKNOWLEDGEMENTS

This work was supported by “shuangzhi” programme of Sichuan Agriculture University (No 00170723) and Key Project Foundation of Sichuan Province Education Bureau (No10ZA040). We wish to express our warm and sincere thanks to the Weidong Zang and Lishan Wang which come from Shanghai Jiaotong University. Their ideas and help had a remarkable improvement on our research and the authors wish it to be known that, in their opinion, Weidong Zang and Lishan Wang should be regarded as joint First Authors.

REFERENCES

Abe H, Urao T, Ito T, Seki M, Shinozaki K,Yamaguchi-Shinozaki K (2003). Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell. 15(1): 63-78.

Buchanan CD, Lim S, Salzman RA, Kagiampakis I, Morishige DT, Weers BD, Klein RR, Pratt LH, Cordonnier-Pratt MM, Klein PE,Mullet JE (2005). Sorghum bicolor's transcriptome response to dehydration, high salinity and ABA. Plant Mol. Biol., 58(5): 699-720.

Chinnusamy V, Schumaker K, Zhu JK (2004). Molecular genetic perspectives on cross©\talk and specificity in abiotic stress signalling in plants. J. Exp. Bot., 55(395): 225.

Ding W, Mouzaki M, You H, Laird JC, Mato J, Lu SC, Rountree CB (2009). CD133+ liver cancer stem cells from methionine adenosyl transferase 1A-deficient mice demonstrate resistance to transforming

growth factor (TGF)-beta-induced apoptosis. Hepatology. 49(4): 1277-1286.

Gao JP, Chao DY, Lin HX (2007). Understanding abiotic stress tolerance mechanisms: recent studies on stress response in rice. J. Integr. Plant Biol., 49(6): 742-750.

Guillaumot D, Guillon S, Deplanque T, Vanhee C, Gumy C, Masquelier D, Morsomme P, Batoko H (2009). The Arabidopsis TSPO-related protein is a stress and abscisic acid-regulated, endoplasmic reticulum-Golgi-localized membrane protein. Plant J., 60(2): 242-256.

Guillaumot D, Guillon S, Morsomme P, Batoko H (2009). ABA, porphyrins and plant TSPO-related protein. Plant Signal Behav., 4(11): 1087-1090.

Hazen SP, Wu Y, Kreps JA (2003). Gene expression profiling of plant responses to abiotic stress. Functional & integrative genomics. 3(3): 105-111.

Hong F, Breitling R (2008). A comparison of meta-analysis methods for detecting differentially expressed genes in microarray experiments. Bioinformatics. 24(3): 374-382.

Hong F, Breitling R, McEntee CW, Wittner BS, Nemhauser JL, Chory J (2006). RankProd: a bioconductor package for detecting differentially expressed genes in meta-analysis. Bioinformatics. 22(22): 2825-2827.

Huang da W, Sherman BT, Lempicki RA (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc., 4(1): 44-57.

Huang PM, Chen JY, Wang SJ (2009). Tissue-specific regulation of rice molybdenum cofactor sulfurase gene in response to salt stress and ABA. Acta Physiologiae Plantarum., 31(3): 545-551.

Hunter S, Apweiler R, Attwood TK, Bairoch A, Bateman A, Binns D, Bork P, Das U, Daugherty L, Duquenne L, Finn RD, Gough J, Haft D, Hulo N, Kahn D, Kelly E, Laugraud A, Letunic I, Lonsdale D, Lopez R, Madera M, Maslen J, McAnulla C, McDowall J, Mistry J, Mitchell A, Mulder N, Natale D, Orengo C, Quinn AF, Selengut JD, Sigrist CJ, Thimma M, Thomas PD, Valentin F, Wilson D, Wu CH, Yeats C (2009). InterPro: the integrative protein signature database. Nucleic Acids Res., 37(Database issue): D211-215.

Jayasekaran K, Kim KN, Vivekanandan M, Shin JS, Ok SH (2006). Novel calcium-binding GTPase (AtCBG) involved in ABA-mediated salt stress signaling in Arabidopsis. Plant cell reports. 25(11): 1255-1262.

Jensen MK, Kjaersgaard T, Nielsen MM, Galberg P, Petersen K, O'Shea C, Skriver K (2010). The Arabidopsis thaliana NAC transcription factor family: structure-function relationships and determinants of ANAC019 stress signalling. Biochem. J., 426(2): 183-196.

Kang JY, Choi HI, Im MY, Kim SY (2002). Arabidopsis basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. Plant Cell. 14(2): 343-357.

Kirch HH, Schlingensiepen S, Kotchoni S, Sunkar R, Bartels D (2005). Detailed expression analysis of selected genes of the aldehyde dehydrogenase (ALDH) gene superfamily in Arabidopsis thaliana. Plant Mol. Biol., 57(3): 315-332.

Kotchoni SO, Kuhns C, Ditzer A, Kirch HH, Bartels D (2006). Over-expression of different aldehyde dehydrogenase genes in Arabidopsis thaliana confers tolerance to abiotic stress and protects plants against lipid peroxidation and oxidative stress. Plant Cell Environ., 29(6): 1033-1048.

Kwon YR, Lee HJ, Kim KH, Hong SW, Lee SJ, Lee H (2008). Ectopic expression of Expansin3 or Expansinbeta1 causes enhanced hormone and salt stress sensitivity in Arabidopsis. Biotechnol. Lett., 30(7): 1281-1288.

Sakamoto H, Maruyama K, Sakuma Y, Meshi T, Iwabuchi M, Shinozaki K, Yamaguchi-Shinozaki K (2004). Arabidopsis Cys2/His2-type zinc-finger proteins function as transcription repressors under drought, cold, and high-salinity stress conditions. Plant Physiol., 136(1): 2734-2746.

Smyth GK (2004). Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol., 3: Article 3.

Vinuela A, Snoek LB, Riksen JA, Kammenga JE (2010). Genome-wide gene expression analysis in response to organophosphorus pesticide chlorpyrifos and diazinon in C. elegans. PLoS One. 5(8): e12145.

Xiong L, Zhu JK (2001). Abiotic stress signal transduction in plants: molecular and genetic perspectives. Physiol. Plant., 112(2): 152-166.