Review Paper

Molecular Analysis of Dehydration in Plants

Mohammad Israil Ansari[1]* and Tsan-Piao Lin[2]

[1]Amity Institute of Biotechnology, Amity University, Lucknow Campus, Lucknow, India

[2]Institute of Plant Biology, National Taiwan University, Taipei, Taiwan

Plants respond to survive under water-deficit conditions via a series of physiological, cellular, and molecular processes culminating in stress tolerance. Many drought-inducible genes with various functions have been identified by molecular and genomic analyses in various plants, including a number of transcription factors that regulate stress-inducible gene expression. The products of stress-inducible genes function both in the initial stress response and in establishing plant stress tolerance. In this short review, recent progress resulting from analysis of gene expression during the drought-stress response in plants as well as in elucidating the functions of genes implicated in the stress response are summarized.

Keywords: Dehydration, Gene expression, Regulation, Transcription.

INTRODUCTION

Drought has a major impact on plant growth and development, limiting crop production throughout the world. 28% of the earth's land is too dry for the crop production. The abiotic stresses that include a component of cellular water deficit, usually noted are salinity and low temperature stresses—can also severely limit crop production. Major research efforts are currently directed at understanding the mechanism of plant response to conditions in which water limits plant growth and development in order to identify gene products that confer adaptation to water-deficit stress. Mechanisms of response to water-deficit stress can be measured at many different levels from the whole plant to the molecular level. Since responses are controlled by the plant genome, recent efforts have focused on the molecular response of the plant to water-deficit stress. Better understanding of the genes that are expressed in response to water deficit are needed to characterize fully the mechanisms that permit adaptation to limiting water conditions. Many research programmes have focused on the cellular signalling mechanisms that are activated by water deficit stress (Shinozaki *et al.,* 2003, Xu *et al.,* 2009, Zhao *et al.,* 2009). Several plants, with the greatest emphasis being placed for further understanding of the molecular mechanisms that underlie the plant response to abiotic stress. The extensive molecular studies involving the understanding of the model plants (Arabidopsis and rice) will translate to applications in the improvement of crop growth and production (Zhang *et al.,* 2004).

Several major classes of genes have been noted that are altered in response to water-deficit stress; genes involved in signalling and gene regulation and gene products that are proposed to support cellular adaptation to water-deficit stress are among the most frequently altered in gene expression. Yet, the functions of the majority of the genes with altered expression remain unknown and there are probably more genes yet to be discovered. Microarray analyses, especially for the model plant *Arabidopsis thaliana*, have reached an advanced stage and are contributing to an understanding of the types and quantities of genes that are regulated by water-deficit stress (Seki *et al.,* 2003). In this review we have discuss the plant dehydration stress molecular analysis.

Gene networks involved in drought stress response and tolerance:

Drought stress induces a range of physiological and biochemical responses in plants. These responses include stomatal closure, repression of cell growth and photosynthesis, and activation of respiration. Plants also

---
*Corresponding author Email: ansari_mi@hotmail.com, miansari@amity.edu

respond and adapt to water deficit at both the cellular and molecular levels. The genes with diverse functions are induced or repressed by these stresses (Yamaguchi-Shinozaki and Shinozaki, 2005). Most of their gene products may function in stress response and tolerance at the cellular level. Significantly, the introduction of many stress-inducible genes via gene transfer resulted in improved plant stress tolerance (Zhang *et al.,* 2004; Umezawa *et al.,* 2006a). Recently, a number of stress-inducible genes have been identified using microarray analysis in various plant species. Now, analysing the functions of these genes is critical to further understanding of the molecular mechanisms governing plant stress response and tolerance, ultimately leading to enhancement of stress tolerance in crops through genetic manipulation.

Drought triggers the production of the phytohormone abscisic acid (ABA), which in turn causes stomatal closure and induces expression of stress-related genes. Several drought-inducible genes are induced by exogenous ABA treatment, whereas others are not affected. Indeed, evidence exists demonstrating the presence of both ABA-independent and ABA-dependent regulatory systems governing drought-inducible gene expression (Liu *et al.,* 1998; Yamaguchi-Shinozaki and Shinozaki, 2005). Both cis-acting and trans-acting regulatory elements functioning in ABA-independent and/or ABA-responsive gene expression induced by drought stress have been precisely analysed at the molecular level (Yamaguchi-Shinozaki and Shinozaki, 2005). Results have obtained through transcriptome analysis of drought-inducible gene expression in Arabidopsis and rice using microarrays, including information supporting potential functions of drought-inducible genes in stress response and tolerance.

Transcriptome analysis of drought-inducible genes

Microarray technology employing cDNAs or oligonucleotides is a powerful tool for analysing gene expression profiles of plants exposed to abiotic stresses such as drought, high salinity, or cold, or to ABA treatment. There are two predominant varieties of microarray technology available, the cDNA microarray and the oligonucleotide microarray, the most prominent being the Affimetrix GeneChip (Kreps *et al.,* 2002). A 7000 full-length cDNA microarray was utilized to identify 299 drought-inducible genes, 54 cold-inducible genes, 213 high salinity-inducible genes, and 245 ABA-inducible genes in Arabidopsis (Seki *et al.,* 2002a, b). More than half of these drought-inducible genes were also induced by high salinity and/or ABA treatments, implicating significant cross-talk between the drought, high salinity, and ABA response pathways. In contrast, only 10% of the drought-inducible genes were also induced by cold stress. Thousands of stress-inducible genes were

identified using the Affimetrix GeneChip array containing oligonucleotides representing ~8000 independent Arabidopsis genes (Kreps *et al.,* 2002). In Arabidopsis, transcriptome using the Affymetrix 23 000 ATH1 GeneChip, have generated thousands of transcriptome data points identifying genes expressed in various tissues and under defined growth conditions, stress induction, and phytohormone treatment (Schmid *et al.,* 2005).

Functions of drought-inducible genes:

The products of the drought-inducible genes identified through the microarray analyses in Arabidopsis can be classified into two groups (Shinozaki *et al.,* 2003). The first group includes proteins that most probably function in abiotic stress tolerance. These include molecules such as chaperones, late embryogenesis abundant (LEA) proteins, osmotin, antifreeze proteins, mRNA-binding proteins, key enzymes for osmolyte biosynthesis, water channel proteins, sugar and praline transporters, detoxification enzymes, and various proteases. The second group is comprised of regulatory proteins, i.e. protein factors involved in further regulation of signal transduction and stress-responsive gene expression. These include various transcription factors, protein kinases, protein phosphatases, enzymes involved in phospholipid metabolism, and other signalling molecules such as calmodulin-binding protein (Xu *et al.,* 2009). Many transcription factor genes were stress inducible, suggesting that various transcriptional regulatory mechanisms may function in regulating drought, cold, or high salinity stress signal transduction pathways. These transcription factors could govern expression of stress-inducible genes either cooperatively or independently, and may constitute gene networks in plants.

Stress inducible genes identified in Arabidopsis, rice and other plants can be classified into functional proteins and regulatory proteins (Rabbani *et al.,* 2003). Comparative analysis of stress-inducible genes in Arabidopsis with those in rice revealed a considerable degree of similarity in stress responses between the two genomes at the molecular level. Among the 73 genes identified as stress inducible in rice, 51 have already been reported in Arabidopsis to perform a similar function (Rabbani *et al.,* 2003). These results confirm that rice shares common stress-inducible genes with Arabidopsis, even though these two plants evolved separately more than a million years ago.

Improved drought stress tolerance in plants via gene transfer:

Introduction by gene transfer of several stress-inducible genes has demonstrably enhanced abiotic stress tolerance in transgenic plants (Zhang *et al.,* 2004; Bartels

and Sunkar, 2005; Umezawa *et al.,* 2006a). These particular genes encode key enzymes regulating biosynthesis of compatible solutes such as amino acids (e.g. proline), quaternary and other amines (e.g. glycinebetaine and polyamines), and a variety of sugars and sugar alcohols (e.g. mannitol, trehalose, galactinol, and raffinose).

Genes encoding LEA proteins and heat shock proteins have also been used to improve drought tolerance in transgenic plants. A gene encoding galactinol synthase (GolS), a key enzyme involved in raffinose family oligosaccharide biosynthesis, was introduced to improve drought-stress tolerance in transgenic Arabidopsis (Taji *et al.,* 2002). Prior analyses demonstrate that GolS genes are induced by drought, cold, and ABA. Moreover, expression of the gene encoding raffinose synthase is also induced by drought stress. Additionally, recent metabolome analysis indicated significant accumulation of both galactinol and raffinose under drought stress. Not only metabolites, but also some stress-responsive proteins such as LEAs, have also been implicated in detoxification and alleviation of cellular damage during dehydration. Other studies demonstrate that overexpression of some LEA class genes results in enhanced tolerance to dehydration, although the precise mechanism is still unknown. LEA proteins may also function as chaperone-like protective molecules to combat cellular damage (Umezawa *et al.,* 2006a). Transcription factors have also proven quite useful in improving stress tolerance in transgenic plants, through influencing expression of a number of stress-related target genes (Yamaguchi-Shinozaki and Shinozaki, 2005).

Molecular mechanisms improved stress tolerance:

Regulatory factors, such as protein kinases and enzymes involved in ABA biosynthesis, are also useful for improving stress tolerance by regulating many stress-related genes in transgenic plants. ABA is synthesized de novo primarily in response to drought and high salinity stress. Genes involved in ABA biosynthesis and catabolism were identified based on genetic and genomics analyses (Nambara and Marion-Poll, 2005). It was demonstrated that overexpression of the gene encoding 9-cisepoxycarotenoid dioxygenase (NCED), a key enzyme in ABA biosynthesis, improves drought stress tolerance in transgenic Arabidopsis plants (Iuchi *et al.,* 2001). Cytochrome P450 CYP707A family member was identified as ABA hydroxylase, an enzyme that degrades ABA during seed imbibition and dehydration stress. A T-DNA insertion mutant of CYP707A3, which is the most abundantly expressed gene amongst the four CYP707A members under stress conditions, exhibited elevated drought tolerance with a concomitant reduction in transpiration rate (Umezawa *et al.,* 2006b).

The ABA-activated SnRK2 protein kinase (OST1/SRK2E) functions in the ABA signal transduction pathway controlling stomatal closure (Mustilli *et al.,* 2002; Yoshida *et al.,* 2002). SnRK2 is a member of the SNF1-related PKase family, which contains 10 members in Arabidopsis and rice. SnRK2s are activated by drought, salinity, and ABA (Yoshida *et al.,* 2002). SRK2E/OST1 is involved in stomatal closure, but not seed germination. Another SnRK2,SRK2C, is activated by osmotic stress, salt stress, and ABA treatment (Umezawa *et al.,* 2004). SRK2C is strongly expressed in the root tip, and is involved in the root response to drought stress. SRK2C also functions in transgenic plants to improve stress tolerance, as many of the downstream genes it influences are stress inducible. In addition, SnRK2 protein kinases may activate transcription factors influencing osmotic stress-responsive gene expression.

Regulation of gene expression:

The promoter of a drought-, high salinity-, and cold inducible gene, RD29A/COR78/LTI78, contains two major cis-acting elements, ABRE (ABA-responsive element) and DRE (dehydration-responsive element)/CRT (C-RepeaT), both of which are involved in stress-inducible gene expression (Yamaguchi-Shinozaki and Shinozaki, 1994, 2005). ABRE and DRE/CRT are cis-acting elements that function in ABA dependent and ABA-independent gene expression, respectively, in response to abiotic stress. Transcription factors belonging to the ERF/AP2 family that bind to these DRE/CRT elements were isolated and termed CBF/DREB1 and DREB2 (Yamaguchi-Shinozaki and Shinozaki, 2005). Their conserved DNA-binding motif is A/GCCGAC. The CBF/DREB1 genes are rapidly and transiently induced by cold stress, the products of which activate the expression of target stress-inducible genes (Jaglo-Ottosen *et al.,* 1998; Liu *et al.,* 1998). Overexpression of CBF/DREB1 in transgenic plants increased stress tolerance to freezing, drought, and salt stresses, suggesting that the CBF/DREB1 proteins function in the development of cold-stress tolerance without modification (Liu *et al.,* 1998). Many CBF/DREB1 target genes have been identified using both cDNA and GeneChip microarrays (Fowler and Thomashow, 2002; Maruyama *et al.,* 2004; Vogel *et al.,* 2005). Most of the CBF/DREB1 target genes contain the DRE motif with a conserved (A/G)CCGACNT sequence in their promoter regions. The target gene products of these proteins are consequently involved in establishing stress tolerance. The DREB2 genes are induced by dehydration stress and may activate other genes involved in drought stress tolerance (Liu *et al.,* 1998).

Rice homologues of CBF/DREB1 and DREB2, 10 OsDREB1s and four OsDREB2s, respectively, have been identified based on rice genome sequence analyses. The

function of these genes in stress-inducible gene expression has been demonstrated in rice. Overexpression of OsDREB1A in Arabidopsis revealed a similar function of the rice genes in stress-responsive gene expression and stress tolerance (Dubouzet *et al.,* 2003). Overexpression of OsDREB1 or Arabidopsis DREB also improved drought and chilling tolerance in rice (Ito *et al.,* 2006). These data indicate that similar transcription factors function in abiotic stress tolerance between dicotyledonous and monocotyledonous plants.

Several drought-inducible genes do not respond to either cold or ABA treatment, suggesting the existence of another ABA-independent pathway regulating the dehydration stress response. These genes include ERD1, which encodes a Clp protease regulatory subunit, ClpD. The ERD1 gene is not only induced by dehydration but is also up-regulated during natural senescence and dark-induced senescence (Nakashima *et al.,* 1997). Promoter analysis of the ERD1 gene in transgenic plants indicates that the ERD1 promoter contains cis-acting element(s) involved not only in ABA independent stress-responsive gene expression but also in senescence-activated gene expression. Analysis of the ERD1 promoter further identified two different novel cis-acting elements involved with dehydration stress induction and in dark-induced senescence (Simpson *et al.,* 2003).

CONCLUSION

Transcriptome analyses based on microarrays have provided powerful tools for discovery of stress-responsive gene in various crop plants and tree species. Transgenic plants generated to express antisense or RNAi constructs, as well as T-DNA- or transposon-tagged mutants, were used to analyse the function of these stress-responsive genes based upon phenotypes resulting from loss of function. Moreover, transgenic overexpressors were very useful not only for functional analyses of stress-inducible genes but also for demonstrating improved stress tolerance in these plants generated by gene transfer. Several factors related to the plant response to drought stress have already been shown to be effective for engineering drought tolerance. Such demonstrated success under experimental conditions has encouraged the use of this strategy to engineer drought tolerance in crop species. Since the application of functional genomics approaches, the identification of genes related to drought response has increased, thus fueling this approach for genetic engineering.

Acknowledgements

REFERENCE

Bartels D, Sunkars R (2005). Drought and salt tolerance in plants. Crit. Rev. Plant Sci., 24: 23–58.

Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki Kamaguchi-Shinozaki K, (2003). OsDREB genes in rice, Oryza sativa L, encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J. 33: 751–763.

Fowler SG, Thomashow MF (2002). Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell 14: 1675–1690.

Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K. Yamaguchi-Shinozaki K (2006). Functional analysis of rice DREB1/CBF-type transcription factors involved in coldresponsive gene expression in transgenic rice. Plant Cell Physiol. 47: 141–153.

Iuchi S, Kobayshi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (2001). Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid, a key enzyme in abscisic acid biosynthesis in Arabidopsis. Plant Cell Physiol. 47: 141–153.

Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF (1998). Arabidopsis CBF1 overexpression induces coe genes and enhances freezing tolerance. Science 280: 104–106.

Kreps JA, Wu Y, Chang HS, Zhu T, Wang X, Harper J (2002). Transcriptome changes for Arabidopsis in response to salt,osmotic, and cold stress. Plant Physiol. 130: 2129–2141.

Liu Q, Sakuma Y, Abe H, Kasuga M, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain, separate two cellular signal transduction pathways in drought- and low temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10: 1491–1406.

Maruyama K, Sakuma Y, Kasuga M, Ito Y, Seki M, Goda H, Shimada Y, Yoshida S, Shinozaki K, Yamaguchi-Shinozaki K (2004). Identification of cold-inducible downstream genes of the ArabidopsisDREB1A/CBF3 transcriptional factor using two microarray systems. Plant J. 38: 982–993.

Mustilli AC, Merlot S, Vavasseur A, Fenzi F, Giraudat J (2002). Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. Plant Cell 14: 3089–3099.

Nakashima K, Kiyosue T, Yamaguchi-Shinozaki K, Shinozaki K (1997). A nuclear gene, erd1, encoding a chloroplast-targeted Clp protease regulatory subunit homolog is not only induced by water stress but also developmentally up-regulated during senescence in Arabidopsis thaliana. Plant J. 12: 851–861.

Nambara E, Marion-Poll M (2005). Abscisic acid biosynthesis and catabolism. Annu. Rev. Plant Biol., 56: 165–185.

Rabbani MA, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y, Yoshiwara K, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003). Monitoring expression profiles of rice (Oryza sativa L.)genes under cold, drought and high-salinity stresses, and ABA application using both cDNA microarray and RNA gel blot analyses. Plant Physiol. 133: 1755–1767.

Schimid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Scholkopf B, Weigel D, Lohmann JU (2005). A gene expression map of *Arabidopsis thaliana* development. Nat. Genetics 37: 501–506.

Seki M, Kameiy A, Yamaguchi-Shinozakiz K, Shinozaki K (2003). Molecular responses to drought, salinity and frost: common and different paths for plant protection. Curr. Opin. Biotechnol., 14: 194–199.

Seki M, Narusaka M, Ishida J, Kameiy A, Yamaguchi-Shinozakiz K (2002a). Monitoring the expression profiles of ca. 7000 Arabidopsis genes under drought,cold, and high-salinity stresses using a full-length cDNA microarray. Plant J. 31, 279–292.

Seki M, Ishida J, Narusaka M, Kameiy A, Yamaguchi-Shinozakiz K (2002b). Monitoring the expression pattern of ca. 7000 Arabidopsis genes under ABA treatments using a full-length cDNA microarray. Funct. Integ. Genomics 2: 282–291.

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003). Regulatory

network of gene expression in the drought and cold stress responses. Curr. Opin. Plant Biol., 6: 410–417.

Simpson SD, Nakashima K, Narusaka Y, Seki M, Shinozaki K, Yamaguhci-Shinozaki K (2003). Two different novel cis-acting elements of erd1, a clpA homologous Arabidopsis gene function in induction by dehydration stress and dark-induced senescence. Plant J. 33: 259–270.

Taji T, Ohsumi C, Iuchi S, Seki M, Kasuga M, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K (2002). Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in Arabidopsis thaliana. Plant J. 29: 417–426.

Umezawa T, Fujita M, Fujita Y, Yamaguchi-Shinozaki K, Shinozaki K (2006a). Engineering drought tolerance in plants:discovering and tailoring genes unlock the future. Curr. Opin. Biotechnol., 17: 113–122.

Umezawa T, Okamoto M, Kushiro T, Nambara E, Oono Y, Seki M, Kobayashi M, Koshiba T, Kamiya Y, Shinozaki K (2006b). CYP707A3, a major ABA hydroxylase involved in dehydration and rehydration response in Arabidopsis thaliana. Plant J. 46: 171–182.

Vogel JT, Zarka DG, Van Buskirk HA, Fowler SG, Thomashow MF (2005). Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis. Plant J. 412: 195–211.

Xu ZS, Liu L, Ni ZY, Liu P, Chen M, Li LC, Chen YF, Ma YZ (2009). W55a encodes a novel protein kinase that is involved in

multiple stress responses. J. Integr. Plant Biol. 51: 58-66.

Yamaguchi-Shinozaki K, Shinozaki K (1994). A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell.   6: 251–264.

Yamaguchi-Shinozaki   K, Shinozaki K (2005). Organization of cisacting regulatory elements in osmotic- and cold-stress-responsive promoters. Trends Plant Sci., 10: 88–94.

Yoshida R, Hobo T, Ichimura K, Mizoguchi T, Takahashi F, Alonso J, Ecker JR, Shinozaki K (2002). ABA-activated SnRK2 protein kinase is required for dehydration stress signaling in Arabidopsis. Plant Cell Physiol., 43: 1473–1483.

Zhang JZ, Creelman RA, Zhu JK (2004). From laboratory to field. Using information from Arabidopsis to engineer salt, cold and drought tolerance in crops. Plant Physiol., 135: 615–621.

Zhao CR, Ikka T, Swaki Y, Kobayash Y, Suzuki Y, Hibino T, Sato S, Sakurani N, Shibata D, Koyama H (2009). Comparative transcriptomic characterization of aluminum, sodium chloride, cadmium and copper rhizotoxicities in Arabidopsis thaliana. BMC Plant Biol., 23: 9-23