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CELLULAR AND MOLECULAR MECHANISMS OF THE PLANT WATER STRESS RESPONSE

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ABSTRACT

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Plants respond to water stress by adaptation of the biochemical and physiological processes designed to improve the water status through combined inhibition of water loss by transpiration and more efficient access to the supplies of soil water. The biochemical and physiological reactions are regulated by precise molecular switches operating in a coordinated fashion Regulation is achieved at the level of gene expression (up- and down-regulation of transcripts), translational modification, modulation of the type and amount of intracellular solutes, and changes in osmolytes and phytohoimonal levels It is accomplished in an organ- and tissue-specific way

This review aims to summarize the recent advances in the understanding of the cellular and molecular mechanisms of the water stress response *inplanta.* The relationship of water stress to regulation of gene expression is accentuated, and the putative function of some of the better characterized water stress-upregulated gene products is outlined. Future approaches directed toward characterization of the molecular mechanisms underlying the physiological responses to water stress are discussed, addressing the use of recombinant DNA techniques as a tool that may contribute to selection schemes in breeding programs for drought tolerance.

Key words: Abscisic acid; Drought stress; Osmotic stress; Water deficit; Water' stress.

ИЗВОД

Станковиќ, Б. (1996). Клеточни и молекуларни механизми како одговор на растителниот воден стрес. Екол. Зашт. Живот. Сред., Том 4, Бр. 1, Скопје.

Во услови на суша растенијата реагираат со прилагодување на биохемиските и физиолошките процеси со цел да го подобрат водниот статус преку комбинирано смалување на загубите на вода низ транспирација, како и со поефикасен пристап до вода во почвата. Биохемиските и физиолошките процеси се регулирани со прецизни молекуларни механизми со координирана функција Регулацијата се остварува на ниво на експресија на гени (преку зголемено и смалено ниво на транскрипција), транслациони модификации, како и прилагодување на видот и количината на осмотски активни супстанции и фитохормони, а се остварува на ниво на органи и ткива

Цел на овој прилог е да ги сумира новите сознанија сврзани со молекуларните механизми коишто се јавуваат при услови на воден стрес кај растенијата. Посебно е нагласен односот на водениот стрес со нивото на експресија на гени. Објаснети се предложените функции на подобро карактеризираните протеини коишто се синтетизираат во услови на воден стрес. Дискутирани се идните можни пристапи кон подобро запознавање на молекуларните механизми коишто ги диктираат физиолошките реакции во услови на воден стрес, потенцирајќи ја употребата на рекомбинантни ДНК техники како средство коешто ќе придонесе за креирање на поефективни селекциони шеми во селекцијата на растенија отпорни на суша.

Клучни зборови: абсцисична киселина; воден дефицит; воден стрес; осмотски стрес; сушен стрес.

INTRODUCTION

Water stress triggered by changes in the external water potential is an environmental commonplace in the life of a higher plant. Plants subjected to water stress (drought stress, osmotic stress, water deficit) lose water from the stem and leaf tissues, and respond with a set of bio-

chemical and physiological adaptations. Initial phenotypic responses involve stomatal closure followed by changes in stomatal distribution (Mansfield and Atkinson 1990) and altered growth equilibrium in favor of the root *vs* the shoot The aim of these processes is to reduce water loss through combined increase in reduced transpiration and water uptake. These phenotypical manifestations result from a new set of organ- and tissue-specific processes, involving increased amount of solutes in the root (Sharp and Davies 1979), changes in the hydraulic conductivity coefficient, changes in cell elasticity (Fiscus 1975; Steudle and Zimmermann 1977), and cell enlargement (Hsiao 1973). Drought decreases cuticular transpiration in seedlings (Bengtson et al. 1978) and increases plasma membrane permeability (Quartacci and Navari-Izzo 1992). It reduces leaf area and protoplast volume (Saradadevi et al. 1996) and decreases photosynthesis reorganizing the photosystem II (PSII), through increased phosphorylation of the PSII core coupled with increased Dl protein synthesis (Giardi et al. 1996). Some plants switch from C_3 to CAM photosynthetic pathway as a means of increasing water use efficiency.

Abiotic stresses such as water deficit, heat stress, desiccation, high light intensity, high salinity, and chilling (cold stress) exhibit commonalities both in terms of the mode of action and the triggered response. They lower the water potential, i.e. decrease the amount of exo-and endogenously-accessible water. All of them share common physiological responses, whose underlying molecular mechanisms might be common: elevation of same mRNA transcripts in response to multiple a biotic stresses has been widely demonstrated (Heikkila et al. 1984; Claes et al. 1990; Neven et al. 1993; Urao et al. 1994). This implies a complexity of the response and existence of overlapping physiological processes resulting from interactions of the loss of water (due to lowered water potential of the soil) with other environmental factors that influence action of stomata, dynamics of osmotically-active substances, etc. Even though interaction and cross-talk with other stress-induced stimulus-response signaling pathways is likely, the plant is capable of distinguishing and elevating a specific (sub) set of genes in response to a range of environmental stimuli. This review focuses on the cellular and molecular responses triggered with water stress, without addressing the possible effects of the interaction of water deficit with other environmental variables.

CELLULAR ADJUSTMENTS TO OSMOTIC STRESS

The cell response to water stress is a result of subtle intracellular molecular strategies designed to minimize water loss, through accumulation of osmoprotectants such as low molecular-weight osmolytes, sugar alcohols, certain amino acids (mainly proline), and Gly betaine (Greenway and Munns 1980; Yancey et al. 1982). It entails a cascade initiated with the perception of stress, transduction of the stress signal, its modulation and amplification, leading to tissue-specific transcriptional and translational regulation.

Little is known about the stimulus-response chain during osmotic stress, which is initiated with net influx of ions causing reduced concentration of free water within the cell. No receptors for water deprivation have been discovered, and it is unclear through what mechanism can a physical phenomenon (i.e. water loss) be transducer into a biochemical response. It has been suggested that the initial detection of the osmotic stress occurs through plasma membrane perturbations, caused by turgor pressure loss (Skrivei and Mundy 1990). Such perturbations could lead to activation of stretch-activated ion (Ca^{2+} ?) channels, initiating a cascade of cytosolic signal transduction involving kinases and resulting in gene activation. A sufficient amount of experimental evidence exists to support this model. Osmotic stress influences lipid metabolism and lipid/phospholipid metabolism through changes in per oxidation levels (Olsson et al. 1996). Such changes in lipid composition could drive the changes in plasma membrane permeability (Quartacci and Navari-Izzo 1992). Osmotic stress also causes depolarization of the plasma membrane (Ishikawa et al. 1983), a process dependent on ion pumping. Under conditions of water deficit, the cell regulates its osmotic potential through ion compartmentalization. This process takes place at the expense of the H^+ electrochemical gradient, which is maintained through action of plasma membrane- and tonoplast-localized ion pumps, ion carriers, and

H^+-ATPases. Such electrochemical response involves redistribution of ions, and intracellular calcium influx, resulting in activation of a signal transduction pathway involving second messengers - calmodulin, Ca^{2+}/calmodulin regulated protein kinases, calcium dependent protein kinases (CDPKs), and/or inositol phospholipids. Osmotic stress induces the phosphorylation activity of a calcium-dependent protein kinase in maize and sorghum roots (Pestenacz and Erdei 1996), and rapidly increases the concentration of intracellular Ca^{2+}, a process which is probably mediated by phosphoinositides (Lynch et al. 1989). Thus the link between drought-induced plasma membrane perturbations and gene expression could be accomplished through a phosphorylation-based signaling cascade.

Phytohormones are involved in the drought stress response. Evidence accumulated over the past few decades suggests a significant physiological role for abscisic acid (ABA), a "generalized stress hormone", in the water stress response. Water deficit rapidly (within 30 min) induces synthesis of this sesquiterpene from a preexisting intracellular pool of xanthophylls, followed by preferential accumulation the root, particularly at the root tips (Schnall and Quatrano 1992). The ABA synthesis is probably dependent on plasma membrane-localized pres-sure-sensitive receptors or ion channels, and requires novel gene expression (references in Guerrero et al. 1990). Following water stress both its infra-plant redistribution and *de novo* synthesis (Wright and Hiron 1972) have been documented. Conversely, elevated ABA levels induce expression of numerous genes (cf. recent comprehensive reviews on the mechanisms of ABA-regulated gene expression - Skriver and Mundy 1990; Chandler and Robertson 1994) ABA induces K^+ and Ca^{2+} fluxes in guard cells causing stomatal closure and regulating water loss (Koornneef et al. 1989). However, despite the burgeoning literature addressing its mechanism of action at the cellular level (Hetherington and Quatrano 1991), it is still unclear where in the drought stress stimulus-response chain lies its role. Rapid release of ABA from intracellular stores following stress could be one of the inital steps in the signal transduction cascade. Drought-induced changes in the photosystem (Giardi et al, 1996) reduce the stromal pH (Cowan et al. 1982), which might lead to release of the chloroplast-compartmentalized ABA into the cytosol. There it could act upon an intracellular receptor initiating a cascade of signal transduction, or could be directly imported into the nucleus triggering gene activation.

Water deficit reduces the amounts of the plant growth regulators cytokinin and gibberellin (Guerrero and Mullet 1988), possibly through ABA-mediated antagonistic action on gibberelic acid-induced gene expression (Chandler and Robertson 1994). The eventual role of the phytohormone ethylene in the water-str ess response is unclear. Whilst increased ethylene production following drought stressed wheat leaves has been reported (Apelbaum and Yang 1981), such observations might be due to the experimental conditions, since the response in detached leaves significantly differs from that observed in intact plants (Morgan et al. 1990). Further investigations using intact plants should be revealing.

GENE EXPRESSION IN RESPONSE TO WATER

Recent studies have shown that a large set of genes becomes up regulated under osmotic stress. It is plausible that some of the newly-synthesized gene products play an instrumental role in the control of the short- and long-term responses to drought, and are involved in elevated tolerance to dehydration through protection of the cellular structure, control of the accumulation of ions, water uptake, and protein turnover. Hence, efforts have been directed toward the isolation and characterization of water stress-inducible genes in the belief that such knowledge might indicate adaptive mechanisms That will have agronomic value (Bray 1993). The current opinion favors a biphasic model of gene regulation following stress occurrence: the first stage of the response entails induction of "early" transcripts, implicated in the perception and the transduction of the stress signal; it is followed by a longer-lasting second stage that involves synthesis of "late" transcripts involved in stress tolerance, assumption of a new homeostatic cellular condition and recovery of the "normal" metabolism. A host of water stress-induced genes have been cloned and sequenced, revealing details on the molecular basis of the water

stress response. Tissue-, organ-, and developmental pattern of expression for some of these genes have been determined. Predicted amino acid sequences suggest functional roles, which can be tested with appropriately designed biochemical and physiological assays. Clones isolated in these studies show sequence homologies to genes associated with plant responses to cell damage: ubiquitin (Borkird et al, 1991), alcohol dehydrogenase (de Bruxelles et al. 1996), heat shock proteins (Heikkila et al. 1984; Almoguera et al. 1993), and proteinase inhibitors (Downing et al. 1992). Other clones encode for polypeptides that fall into the categories of: pathogenesis-related proteins (Singh et al. 1989); proteins involved in cell wall elasticity and/or reinforcement (Iraki et al. 1989; Chang et al. 1996); proteins involved in osmotic regulation (Yamaguchi-Shinozaki et al. 1992); desiccation survival (Close et al. 1989); signal transduction (Urao et al. 1994); RNA-binding (Mortenson and Dreyfuss 1989); and lipid transfer (Plant et al. 1991). Water deficit stimulates the expression of putative ion or water-channel proteins (Guerrero et al. 1990; Yamaguchi-Shinozaki et al. 1992). It is conceivable that accumulation of channel proteins in the tonoplast and the plasma membrane will have an instrumental role in movement and sequestering of water, ions and solutes between the vacuole and the cytoplasm, adjusting the osmotic potential over an extended time frame, perhaps as a component of the tolerance mechanism. In some reports where novel water stress-induced genes that show very small degree of similarity to known sequences in the GenBank database have been identified (Piatkowski et al. 1990; Chen et al. 1996), the functions have largely remained unknown, and can only be speculated based upon the partial degrees of homology with cloned and sequenced genes from other organisms (Chang et al. 1996). Where examined, the water stress-induced genes show organ- and tissue-specific pattern of expression, which implies their specific functions. Drought-induced *sa*/T mRNA in rice rapidly accumulates in roots and sheaths, but not in the leaf lamina (Claes et al. 1990). Osmotin accumulates in vacuoles of stressed plants (Singh et al. 1989). The water deficit-inducible tomato protein TMA SN1 (similar to eukaryotic chromosomal proteins) is localized primarily in the nucleus where it could regulate gene expression influencing DNA super coiling and chromatin structure (Iusem et al. 1993).

Since the recognition of ABA as an important component in the water stress stimulus-response pathway, significant efforts have been devoted to characterization of genes induced in vegetative parts of plants by external ABA application to unstressed plants (Skriver and Mundy 1990). The rationale behind these studies has been that these genes could be involved either in the perception of the stress (through ABA as a mediator) or in the adaptation mechanisms. Indeed, treating plants with exogenous ABA frequently hardens them against stress, suggesting that the ABA-regulated gene products might be involved in stress tolerance. Correlation between ABA levels and changes in levels of certain mRNA transcripts has been demonstrated. Since in many studies the function of the ABA-regulated genes has not been determined, they have been grouped and classified according to various criteria: (/) develo-mentally-regulated *(/ea,* late embryogenesis abundant genes); expression level-related *(rab,* genes responsive to ABA); environmentally-regulated *(dhn,* dehydration-induced); and amino acid-labeling pattern *[Em,* early methionine labeled). The observations of gene activation do not necessarily prove that water stress-induced ABA is the sole factor that is responsible for gene expression. Frequently observed inconsistencies between changes in ABA levels and levels of ABA-upregulated transcripts under different stress conditions (i.e. in interaction with environmental stresses other than drought) suggest that additional factors modulate the ABA-induced gene expression. Even though ABA elevates overall transcriptional rate, through an unknown mechanism it selectively up- or down-regulates levels of particular mRNAs (DeLisle and Crouch 1989). Furthermore, the ABA-induced transcriptional response is tissue-, gene-, and developmental stage-specific: some ABA-upregulated genes in vegetative tissues apparently do not require ABA for expression in seeds (Chandler and Robertson 1994).

One group of genes upregulated following ABA treatment has generically been named with the acronym/»^ (responsive to ABA). Both changes in ABA levels and expression of *rab* genes have been correlated with water stress tolerance (Bray 1988; Gomez et al. 1988; Mundy

and Chua 1988; Singh et al. 1989). The amino acid sequences of the RAB polypeptides are highly hydrophilic with a high content of uncharged and hydroxyl Ted amino acids. Some RAB proteins might have regulatory functions through RNA-binding capacity (Mundy and Chua 1988).

Another group of ABA-induced gene products falls into a functionally similar' category of proteins designed to preserve the cell structure during dehydration. These are the late embryogenesis abundant (LEA) proteins that accumulate as osmoprotectants in the developing embryos of higher plants, enabling the protoplastic dehydration during seed maturation. Based upon the predicted amino acid sequence similarities and the determined intracellular compartmentalization, six different groups of *lea* genes have so far been characterized (Dure 1993), and functions have been predicted for some of the *lea* gene products: group 1 LEA proteins are predicted to have water-binding capacity; group 2 LEA proteins have suggested chaperone function, enabling them to preserve protein structure; group 3 and group 5 LEA proteins are most likely involved in the sequestration of ions during water deficit; group 4 LEA proteins have the potential of replacing water in the membrane to preserve its structure (Dure 1993). Recent findings indicate correlation of the amount of LEA protein with the degree of water' stress tolerance (Reid and Walker-Simmons 1993; Xu et al. 1996).

The water stress effect on gene action is not limited only to synthesis of new transcripts. Drought stress and ABA application modulate the expression of various constitutively expressed genes (Chen et al. 1996), and are also capable of down regulating transcription. For example, genes whose products are involved in photosynthesis *{cab* and *rbcS)* are negatively-(down-) regulated by ABA in water-stressed tomato leaves (Bartholomew et al. 1991).

PROSPECTS

Since our understanding of the molecular mechanisms involved in the water stress response is in its initial stage, the future prospects are numerous and exciting. The transcriptional induction of a given gene does not have to be causally related to adaptation to stress, i.e. to drought tolerance. In some cases gene activation could be a consequence of the experienced stress and a part of the common stress response. It is also important to recognize the differences in the experimental protocols used in many of the above studies. The materials examined range from protoplasts of different origin through detached leaves to intact plants. Application of osmotic stress has typically involved rapidly imposed dehydration, sudden application of high osmoticum (e.g. polyethylene glycol), or abrupt increase in salt (NaCl) concentration. These treatments could result in arte factual effects of the rapidly imposed laboratory stresses, frequently imposed over brief periods of several hours, as opposed to the water' deficit which in the field develops gradually, over an extended period of time (Radin 1993; Chen et al. 1996). Leone et al. (1994) showed that potato cell suspension cultures synthesize different sets of polypeptides when subjected to rapid *vs* gradually imposed water stress. Thus some of the isolated and reported genes might be largely representative of a common stress-induced response, rather than being specifically associated with the water stress response, let alone adaptation or stress tolerance.

The discovery and cloning of water stress-regulated genes through differential cloning, coupled with construction of transgenic plants (either carrying antisense constructs or over-expressing the drought up-regulated proteins), could be a powerful tool to reveal gene and protein function. A recent report demonstrated that rice plants transformed with a late embryogenesis abundant (LEA) protein gene from barley *{HVAT)* became more tolerant to water stress (Xu et al. 1996). Identification of common and specific response elements which confer short-term or long-term enhancement of transcript expression can lead to the construction of transgenic plants capable of making water stress-related proteins over a more permanent time-frame. Elucidation of ABA-responsive promoter regions has successfully begun, revealing a ax-acting ABA-response element ABRE in the promoter region of several ABA-regulated genes (Yamaguchi -Shinozaki et al. 1990; Chandler and Robertson 1994). Further work should delineate the *cis*- and *trans*- acting factors con-

trolling the expression of the *lea* and the *rab* genes. Some of the *lea* and *rab* genes already exhibit the potential for use as a molecular tool for genetic crop improvement toward osmotic stress tolerance (Xu et al. 1996),, Similarly, over expression of the water stress-induced osmotin in potato proved to be effective against fungal disease (Liu et al. 1994). Differential display of genes preferentially expressed in water stress-tolerant *vs* susceptible species and varieties might provide insight into the nature of the proteins involved in stress tolerance. *In situ* mRNA hybridization and use of reporter genes (GUS, GFP) under the control of water stress-inducible promoters can confirm the localization and tissue specificity of the discovered genes. Screening for water stress-response mutants, with complementation of the already available ABA mutants, will help dissect the individual steps in the mechanisms of osmotic stress response and tolerance.

Transcript processing, regulation of mRNA stability, translational control, protein activity, and protein turnover are additional factors that regulate the function of the stress-induced gene products. Even though water deficit results in overall down regulation of translation, i.e. suppression of the synthesis of constitutive cellular proteins (Hanson and Hitz 1982; Bartholomew et al. 1991), the protein stability and protein turnover are a function of two dynamic opposite processes. Drought induces synthesis of both chaperons and proteins inhibitors (which enhance stability of other proteins), and proteases and ubiquitin (which cause degradation of proteins that become denatured during water loss). Simultaneous existence of two counteracting mechanisms (activation and inhibition of the translation of specific proteins) may preferentially direct the synthesis of ± stress proteins[2], and requires the presence of translational regulatory factors that have yet to be identified. A recently identified water stress-upregulated RNA-binding protein from maize (Mortenson and Dreyfuss 1989) might be a candidate for such post-transcriptional regulation. If the primary effects of water stress at molecular level are elucidated, and isolated gene products become functionally correlated to adaptation to water stress, it might be possible to use recombinant DNA technology to alter specific biochemical and physiological processes, increasing the tolerance to drought. A combined approach using physiological, molecular biology and mutational (molecular genetics) studies will likely lead to the development of more efficient methods of plant protection and water stress tolerance.

REFERENCES

Almoguera, C, Coca M.A., Jordano, J. (1993). Tissue-specific expression of sunflower heat shock proteins in response to water stress. *Plant Journal A:* 947-958.

Apelbaum, A., Yang, S.F. (1981). Biosynthesis of stress ethylene induced by water deficit. *Plant Physiology* 68: 594-596.

Bartholomew, D.M., Bartley, G.E., Scolnik P. A. (1991). Abscisic acid control of rbcS and cab transcription in tomato leaves. *Plant Physiology*'96: 291-296.

Bengtson, C., Larsson, S., Liljenberg, C. (1978). Effects of water stress on cuticular transpirationrate and amount and composition of epicuticular wax in seedlings of six oat varieties. *Physiologia Plantarum* 44: 319-324.

Borkird, C, Simeons, C., Villarroel, R., van Montagu M. (1991). Gene expression associatedwith water stress adaptations of rice cells and identification of two genes as hsp70 andubiquitn.*Physiologia Plantarum* 82: 449-457.

Bray, E.A. (1988). Drought and ABA-induced changes in polypeptide and mRNA accumulation in tomato leaves. *Plant Physiology*-88: 1210-1214.

Bray, E.A. (1993). Molecular responses to water deficit. *Plant Physiology* 103: 1035-1040.

Chandler, P.M., Robertson, M. (1994). Gene expression regulated by abscisic acid and its relation to stress tolerance .*Annual Review of Plant Physiology and Plant Molecular Biology*45: 113-141.

Chang, S,,, Puryear, J.D., Dias, D.L., Funkhouser, E.A., Newton, R.J., Cairney, J. (1996). Gene expression under water deficit in loblolly pine (*Pinustaeda):* Isolation

and characterization of cDNA clones, *Physiologia Planiarum* 97: 139-148., Chen, J., Reinisch, A.J., Adair, L.S., Newton, R.J., Funkhouser, E.A., Cairney, J, (1996). Isolation,sequence and expression analysis of two water deficit related cDNA clones from thehalophyte *Atriplex canes-cens* (saltbush). *Physiologia Plantarum* 96: 401-410.

Claes, B,„ Dekeyser, R., Villaroel, R,„ van din Bulcke, M,„ Bauw, G., van Montagu, M., Caplan, A.(1990). Characterization of a rice gene showing organ-specific expression in response tosalt-stress and drought. *Plant Cell:* 19-27.

Close, T.J., Kortt, A.A., Chandler, P.M. (1989), A cDNA-based comparison of dehydrationinduced proteins (dehydrins) in bailey and com. *Plant Molecular Biology* 13: 95-108.

Cowan, I.R., Raven, J.A., Hartung, W., Farquhar, G.D. (1982). A possible role for abscisic acid incoupling stomatal conductance and photosynthetic carbon metabolism in leaves. *Australian Journal'of Plant Physiology'9:* 489-498.

de Bruxelles, G.L., Peacock, W.J., Dennis, E.S., Dolferus, R. (1996). Abscisic acid induces thealcohol dehydrogenase gene in *Arabidopsis. Plant Physiology* 111: 381-391.

DeLisle, A.J., Crouch, M.L. (1989). Seed storage protein transcription and mRNA levels in *Brassica napus* during development and in response to exogenous abscisic acid. *PlantPhysiology* 91: 617-623.

Downing, W.L., Mauxion, R., Fauvarque, M„-O., Reviron, M.-P., de Vienne, D., Vartanian, N.,Giraudat, J. (1992). A *Brassica napus* transcript encoding a protein related to the Kunitz protease inhibitor family accumulates upon water stress in leaves, not in seeds *Plant Journal* 2*:* 685-693.

Dure, L. Ill (1993). Structural motifs in Lea proteins. In: *Plant Responses to Cellular Dehydration during Environmental Stress. Current Popics in Plant Physiology,* Close T.J. and Bray E.A., eds, Vol. 10, pp 91-KB.

Fiscus, E.L. (1975). The interaction between osmotic-and pressure-induced water flows in plantroots. *Plant Physiology*55: 917-922.

Giardi, M.T., Cona, A., Geiken, B,„ Kucera, T., Masojidek, J., Mattoo, A.K. (1996). Long-term drought stress induces structural and functional reorganization of photo system II *Planta* 199: 118-125.

Gomez, J., Sanchez-Martinez, D., Stiefel, V., Rigau, J., Puigdomenech, P., Pages, M. (1988). Agene induced by the plant hormone abscisic acid in responses to water stress encodes aglycine-rich protein. *Nature 3U: 262-26 A,*

Greenway, H,„ Munns, R. (1980). Mechanisms of salt tolerance in nonhalophytes., *Annual Review of Plant Physiology* 31: 149-190.

Guerrero, F.D., Mullet, J.E. (1988). Reduction of turgor induces rapid changes in leaf translatable RNA. *Plant Physiology* 88: 401-408.

Guerrero, F.D., Jones, J.T., Mullet, J.E. (1990). Turgor-responsive gene transcription and RNAlevels increase rapidly when pea shoots are wilted. Sequence and expression of three inducible genes, *Plant Molecular Biology*T5: 11-26.

Hanson, A.D., Hitz, W.D. (1982). Metabolic responses of mesophytes to plant water deficits. *Annual Review of PlantPhysiology*33: 163-203.

Heikkila, J., Papp, J.E.T., Schultz, G.A, Bewley, J.D. (1984). Induction of heat shock pr oteinmessenger RNA in maize mesocotyls by water stress, abscisic acid, and wounding*Plant Physiology* 76: 270-274.

Hetherington, A.M., Quatrano, R.S. (1991). Mechanisms of action of abscisic acid at the cellular level. *New Phytologist* 119: 9-32.

Hsiao, T.C. (1973). Plant responses to water stress. *Annual Review of Plant Physiology* 24:519-570.

Iraki, N.M., Bressan, R.A., Hasegawa, P.M., Carpita, N.C. (1989). Alteration of the physical and chemical structure of the primary cell wall of growth-limited plant cells adapted to osmotic stress. *Plant Physiology* 91: 39-47.

Ishikawa, H., Abou, S,„ Ohta, E., Sakata, M. (1983). Effects of periodic-and transient-yroots. *Plant and Cell Physiology* 24: 1129-1135.

Iusem, D.N., Bartholomew, D.M., Hitz, W.D., Scolnik, P.A. (1993). Tomato *(Lycopersiconesculentum)* transcript induced by water deficit and ripening. *Plant Physiology* 102:1353-1354.

Koornneef, M., Hanhart, C.J., Hilhorst, H.W.M., Karssen, CM. (1989). In vivo inhibition of seed development and reserve protein accumulation in recombinants of abscisic acid biosynthesis and responsiveness mutants in *Arabidopsis thaliana. Plant Physiology* 90:463-469.

Leone, A., Costa, H., Tucci, M., Grfflo, S. (1994). Comparative analysis of short- and longtermchanges in gene expression caused by low water potential in potato *(Solanum tuberosum)* cell-suspension culture. *Plant Physiology* 106: 703-712.

Liu, D., Raghothama, KG., Hasegawa, P.M., Bressan, R.A (1994). Osmotin overexpression inpotato delays development of disease symptoms. *Proceedings of the National Academy of Sciences USA* 91: 1888-1892.

Lynch, J, Polito, V.S., Lauchli, A. (1989). Salinity stress increases cytoplasmic Ca activity inmaize root protoplasts. *Plant Physiology*-90: 1271-1274,

Mansfield, TA,,, Atkinson, C.J. (1990). Stomatal behaviour in water stressed plants, In: *Stress Responses in Plants: Adaptation and Acclimation Mechanisms,* Alscher R.G. andCumming J.R., eds., Wiley-Liss Inc., New York, pp 241-264.

Morgan, P.W., He C.-J., De Greef, J.A., De Proft, M.P. (1990). Does water deficit stress promote ethylene synthesis by intact plants? *Plant Physiology* 94: 1616-1624.

Mortenson, E., Dreyfuss, G. (1989). RNP in maize protein. *Nature* 337: 312.

Mundy, J., Chua, N.-H. (1988). Abscisic acid and water-stress induce the expression of a novelrice gene. *EMBO Journall:* 2279-2286.

Neven, L.G., Haskell, D.W., Hofig, A., Li Q,-B., Guy, C.L. (1993). Characterization of a spinach gene responsive to low temperature and water stress. *Plant Molecular Biology* 21*:* 291-305.

Olsson, M., Nilsson, K., Liljenberg, C., Hendry, A.F. (1996). Drought stress in seedlings: lipidmetabolism and lipid per oxidation during recovery from drought in *Lotus corniculatus* and *Cerastium fontanum, Physiologia Plantarum* 96: 577-584.

Pestenacz, A., Erdei, L, (1996). Calcium-dependent protein kinase in maize and sorghum induced by polyethylene glycol. *Physiologia Plantarum* 97: 360-364.

Piatkowski, D., Schneider, K., Salamini, F,,, Bartels, D. (1990). Characterization of five abscisicacid-responsive cDNA clones isolated from the desiccation-tolerant plant *Craterostigma plantagineum* and their relationship to other water-stress genes. *Plant Physiology* 94*:* 1682-1688.

Plant, A.L., Cohen, A., Bray, E.A. (1991), Nucleotide sequence and spatial expression patternof a drought- and ABA-induced gene of tomato. *Plant Physiology* 97*:* 900-906.

Quartacci, M.F., Navari-Izzo, F. (1992). Water stress and free radical mediated changes in sunflower seedlings. *Journal of Plant Physiology* 139: 621-625.

Radin, J.W. (1993). Water relations of cotton in controlled environments and in the field. *InPlant Responses to Cellular Dehydration during Environmental Stress. Current Topics in Plant Physiology,* Close T.J. and Bray E.A., eds, Vol. 10, pp 67-78.

Reid, J.L., Walker-Simmons, M.K. (1993). Group 3 late embryogenesis abundant proteins indesiccation-tolerant seedlings of wheat *(Triticum aestivum* L.). *Plant Physiology* 102:125-131.

Saradadevi, K., Padmasree, K., Raghavendra, A.S. (1996). Correlation between the inhibition ofphotosynthesis and the decrease in area of detached leaf discs or volume/absorbance ofprotoplasts under osmotic stress in pea *(Pisum sativum), Physiologia Plantarum* 96:395-400.

Schnall, J.A, Quatrano, R.S. (1992). Abscisic acid elicits the water-stress response in root hairsof *Arabidopsis thaliana. Plant Physiology*100: 216-218.

Schroeder, J.I., Hedrich, R. (1989), Involvement of ion channels and active transport in

osmoregulation and signaling of higher plant cells, *Trends in Biochemical Sciences* 14:187-192.

Sharp, RE., Davies, W.J. (1979). Solute regulation and growth by roots and shoots of waterstressed maize plants. *Planta* 147: 43-49.

Singh, N.K., Nelson, D.E., Kuhn, D„, Hasegawa, P.M., Bressan, R.A. (1989). Molecular cloningof osmotin and regulation of its expression by ABA and adaptation to low water potential. *Plant Physiology* 90:1096-1101.

Skriver, K, Mundy, J. (1990). Gene expression in response to abscisic acid and osmotic stres *Plant Cell* 2*:* 503-512.

Steudle, E., Zimmermann, U. (1977). Effect of turgor pressure and cell size on the wall elasticityof plant cells*. PlaniPhysiology* 59*:* 285-289.

Urao, T., Mizoguchi, T„, Yamaguchi-Shinozaki, K., Hayashida, N., Shinozaki, K. (1994). Twogenes that encode Ca^{2+}-dependent protein kinases are induced by drought and high-saltstresses in *Arabidopsis thaliana. Molecular and General Genetics* 244: 331-340.

Wright, S.T.C., Hiron, R.W. P. (1972). The accumulation of abscisic acid in plants during wilting and under other stress conditions. In: *Plant Growth Substances,* Can D.J. ed, Springer-Verlag, Berlin, pp 291-298.

Xu, D., Duan, X., Wang, B., Hong, B., Ho, T. - H. D., Wu R. (1996). Expression of a late embryogenesis abundant protein gene, *HVA1,* from barley confers resistance to waterdeficit and salt stress in transgenic rice. *PlantPhysiology* 110: 249-257.

Yamaguchi-Shinozaki, K., Mino, M„, Mundy, J., Chua, N. (1990)., Analysis of an ABA-responsiverice gene promoter in transgenic tobacco. *Plant Molecular Biology* 15: 905-912.

Yamaguchi-Shinozaki, K., Koizumi, M., Urao, S., Shinozaki, K. (1992). Molecular cloning andcharacterization of 9 cDNAs for genes that are responsive to desiccation in *Arabidopsis thaliana*. Sequence analysis of one cDNA clone that encodes a putative transmembrane channel protein. *Plant Cell Physiology* 33: 217-224.

Yancey, P.H., Clark, ME., Hand, S.C, Bowlus, R.D., Somero, G.N. (1982). Living with water stress: evolution of osmolyte system. *Science* 217: 1214-1222.