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WATER STRESS INDUCES THE UP-REGULATION OF A SPECIFIC SET OF GENES IN PLANTS: ALDEHYDE DEHYDROGENASES AS AN EXAMPLE.

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Summary. The deleterious effect of osmotic stress is often caused by the accumulation of reactive molecules e.g. aldehydes. These molecules can cause lipid peroxidation and modifications of proteins and nucleic acids. Aldehydes can be converted to non-toxic carboxylic acids by different aldehyde dehydrogenases (ALDH). ALDHs occur in all organisms implicating their importance in general biological functions. Aldehydes do not only represent toxic molecules but they are also intermediate products in the synthesis of osmolytes which have been shown to be protective molecules in osmotic stress. For this reason a careful balance of aldehydes is required. Evidence emerges that ALDH enzymes are involved in maintaining this balance, and the investigation of the physiological role of plant-ALDHs begins to attract attention. This review tries to summarize the current knowledge of stress-regulated ALDHs in plants. It describes how ALDHs can be used to obtain more stress tolerant plants by overexpressing ALDH genes. ALDH genes have been used in two ways: 1. to obtain increased accumulation of osmolytes e.g. glycine betaine, 2. to detoxify aldehydes.

Key words: Aldehyde dehydrogenase, Genetic engineering, Stress tolerance, Water stress.

Introduction

Availability of water is one of the most important factors, which determine geographical distribution and productivity of plants (Bartels, 2001a). Water stress is perceived

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as water deficit and can occur with different severity (Ramanjulu and Bartels, 2002). A continuation of a mild water deficit leads to drought and even desiccation (loss of most of the protoplasmic free or bulk water). The response and adaptation of plants to such conditions are very complex and highly variable. Being sessile organisms, plants have developed various strategies to acquire stress tolerance. These strategies include changes in metabolic processes, structural changes of membranes, expression of specific genes and production of secondary metabolites (Thomashow, 1994; Shinozaki and Yamaguchi-Shinozaki, 2000; Ramanjulu and Bartels, 2002). To date, there have been many reports on the molecular mechanisms involved in the response of plants to changes in environmental conditions. One important area of molecular studies on water stress has been the identification and characterization of the late-embryogenesis-abundant (LEA) proteins (Ingram and Bartels, 1996). The hydrophilic proteins, present in seeds during maturation and in vegetative plant tissues in response to water stress, have been proposed to protect tissues from stress damage (Ingram and Bartels, 1996).

In extreme stress conditions, key metabolic systems such as photosynthesis are among the most affected. The capacity of the electron transport chain in such conditions exceeds the consumption of reduction equivalents delivered to the stroma side of the thylakoid membranes (Niyogi et al., 1997). Duration of this constraint is harmful to plants, because it triggers the production of reactive oxygen species (ROS), such as hydroxyl radicals, singlet oxygen, superoxide and hydrogen peroxide (Lamb and Dixon, 1997; Bolwell, 1999; Bartels, 2001b). Plants have evolved mechanisms to protect themselves against the accumulation of these molecules (Pastori and Foyer, 2002).

Aldehyde dehydrogenases (ALDH, EC 1.2.1.3) represent a group of enzymes, which may play a role in stress relevant detoxification processes. Here we will review some aspects of plant-ALDH genes under stress conditions and their relative functions associated with abiotic stress tolerance. Aldehydes and their intermediates are common by-products of a number of metabolic pathways (Schauenstein et al., 1977; Bartels, 2001b). They are referred to as a group of highly reactive and often toxic molecules, which can easily attack cellular nucleophiles such as nucleic acids, proteins and carbohydrates (Skibbe et al., 2002). Therefore the removal of aldehydes and their intermediates is essential for cellular survival. ALDHs catalyze the oxidation of toxic aldehydes to their non-toxic corresponding carboxylic acids (Perozich et al., 1999). Various distinct ALDHs have been studied and widely characterized especially in humans (Lindahl, 1992; Yoshida et al., 1998). Limited characterizations have been carried out on the corresponding plant-ALDHs. Often in tandem with alcohol dehydrogenase, ALDHs act in detoxifying a variety of organic compounds, toxins and pollutants. Recently, it has been reported that various plant-ALDH transcripts accumulate in response to environmental stresses (Barclay and McKersie, 1994; Kirch et al., 2001). Understanding the processes by which plant-ALDH activities limit the cellular damage caused by toxic aldehydes may represent a critical protective strategy for surviving osmotic and even oxidative stress in plants.

Aldehyde dehydrogenases (ALDH), multifunctional enzymes

It is not the purpose of this review to cover the whole subject of stress inducible genes. We focus on the up-regulation of ALDH genes under abiotic stress and how they can be used to improve stress tolerance. ALDHs represent a group of enzymes divided in diverse subfamilies with different functions including detoxification, intermediary metabolism, osmotic protection, and NADPH generation (Perozich et al., 1999). ALDH genes are present in genomes of all organisms analyzed to date, implicating the importance of these enzymes in general biological functions. The ALDH superfamily includes the $NAD(P)^+$-dependent enzymes that oxidize a wide spectrum of endogenous and exogenous aldehydes (Lindahl, 1992). ALDHs are divided into classes based on their substrate specificity. Some ALDHs, known as non-specific ALDHs, react with a wide range of substrates and oxidize a variety of aliphatic and aromatic aldehydes. This group includes the cytosolic and mitochondrial tetrameric class 1 and 2 ALDHs and the dimeric class 3 ALDHs. They were reported to be associated with carcinogenesis and genetic disorders in human (Yoshida et al., 1998). Substrate specific ALDHs include the semialdehyde dehydrogenases (SemiALDHs) such as glutamate SemiALDH, succinate SemiALDH, aspartate SemiALDH, 2-amino-adipate-6-SemiALDH and others such as betaine ALDH (BALDH), or phenylacetaldehyde dehydrogenase (Perozich et al., 1999; Sophos et al., 2001). Complete genome sequences of various species revealed 331 ALDH genes of which only eight were found in archaea, 165 in eubacteria and 158 in eukaryota (Sophos et al., 2001). A nomenclature based on sequence similarity has been developed for eukaryotic ALDH genes, and this can be accessed in www.uchsc.edu/sp/sp/alcdbase/aldhcov.html (Vasiliou et al., 1999). Taking a human ALDH1A1 as an example for the nomenclature, ALDH indicates the root; the first digit (1) indicates a family and the first letter (A) a subfamily, while the final number (1) identifies an individual gene within a subfamily as illustrated by Vasiliou et al. (1999) (see Table 1). A complete list of all ALDH sequences known to date along with the evolutionary analysis in eukaryotes is presented by Sophos et al. (2001).

Although ALDHs have been studied extensively in various organisms, the molecular and physiological involvement of these enzymes in plant stress tolerance has to be elucidated. They are proposed to be involved in ROS scavenging processes (Op Den Camp and Kuhlemeier, 1997). During environmental challenges, the generation of ROS leads to extensive cellular damage including lipid peroxidation of cellular membrane (Hasegawa et al., 2000). One of the common by-products of lipid peroxidation is malondialdehyde (MDA), a highly toxic messenger for ROS-induced damage (Esterbauer et al., 1991). It has been proposed that a continuous detoxification of such an aldehyde and its intermediate by relevant ALDHs would reduce the oxidative damage. The resurrection plant *Craterostigma plantagineum* and the desiccation-tolerant moss *Tortula ruralis* are important experimental systems for studying the molecu-

Table 1. Plant-ALDH Genes up-regulated by various abiotic stresses

Gene name	Identity	Source	Stress regulation or putative functions	References
BADH (ALDH10A4)	Betaine aldehyde dehydrogenase	*Amaranthus hypochondriacus*	Induced under osmotic stress and ABA	Legaria et al., 1998
BADH (ALDH10A3)	Betaine aldehyde dehydrogenase	*Atriplex hortensis*	Enhanced osmotic stress tolerance	Xiao et al., 1995
N/A (ALDH10A11)	Betaine aldehyde dehydrogenase	*Avicennia marina*	Induced under osmotic stress	Hibino et al., 2001
BADH (ALDH10A2)	Betaine aldehyde dehydrogenase	*Beta vulgaris*	Improved salt and osmotic stress tolerance	McCue and Hanson, 1992
BADH (ALDH10A6)	Betaine aldehyde dehydrogenase	*Hordeum vulgare*	Response to osmotic stress and ABA	Ishitani et al., 1995
SBALDH (ALDH10A1)	Betaine aldehyde dehydrogenase	*Sorghum bicolor*	Improved drought tolerance	Whitsitt et al., 1997
BADH15 (ALDH10A)	Betaine aldehyde dehydrogenase	*Sorghum bicolor*	Improved drought tolerance	Wood et al., 1996
BADH (ALDH10A7)	Betaine aldehyde dehydrogenase	*Spinacia oleracea*	Improved drought and salt stress tolerance	Weretilnyk and Hanson, 1990
BNBTG26 (ALDH7B3)	Turgor ALDH like protein	*Brassica napus*	Improved drought tolerance	Stroeher et al., 1995
PSCC26G (ALDH7B1)	Turgor Aldehyde dehydrogenase	*Pisum sativum*	Increased water deficit and osmotic stress tolerance	Guerrero et al., 1990
P5cS-1 (ALDH18B1)	Delta 1-pyrroline-5-carboxilate synthase	*Medicago sativa*	Induces salt stress tolerance	Ginzberg et al., 1998

Table 1. Plant-ALDH Genes up-regulated by various abiotic stresses (Continued)

Gene name	Identity	Source	Stress regulation or putative functions	References
P5cS (ALDH18B1)	Delta 1-pyrroline-5-carboxilate synthase	*Oryza sativa*	Improved salt stress tolerance	Igarashi et al., 1997
Pro2 (ALDH18B1)	Delta 1-pyrroline-5-carboxilate synthase	*Solanum esculentum*	Regulation of proline biosynthesis	Maggio et al., 1996a
CAtP5CS (ALDH18)	Delta 1-pyrroline-5-carboxilate synthase	*Arabidopsis thaliana*	Proline synthesis under osmotic stress	Yoshida et al., 1995
tomPro1 (ALDH19)	Gamma-glutamyl-phosphate reductase	*Solanum esculentum*	Regulation of proline biosynthesis	Maggio et al., 1996b
N/A (ALDH11)	Glyceraldehyde-3-P dehydrogenase	*Apium graveolens*	NADPH supply and mannitol biosynthesis	Gao et al., 2000
GapC-Crat (ALDH11)	Cytosolic Glyceraldehyde-3-P dehydrogenase	*Craterostigma plantagineum*	Induced under dehydration and ABA treatment	Velasco et al., 1994
Cp-ALDH	Aldehyde dehydrogenase	*Craterostigma plantagineum*	Induced under dehydration and ABA treatment	Kirch et al., 2001
ALDH21A1	Aldehyde dehydrogenase	*Tortula ruralis*	Induced under desiccation and salt stress	Chen et al., 2002b

lar basis of desiccation tolerance (Phillips et al., 2002). Kirch et al. (2001) reported the molecular characterization of a novel class of plant-ALDHs: Cp-ALDH from *Craterostigma plantagineum* and Ath-ALDH3 from *Arabidopsis thaliana* showing 70% similarity to each other. Transcripts of Cp-ALDH and Ath-ALDH3 accumulate in response to dehydration and ABA-treatment. It was shown that the recombinant Cp-ALDH protein oxidized nonanal, propionaldehyde and acetaldehyde. Furthermore, Chen et al. (2002b) have also characterized a stress-responsive *Tortula ruralis* gene ALDH21A1 described as a novel eukaryotic aldehyde dehydrogenase protein family. ALDH21A1 is most closely related to members of the non-substrate specific ALDH11 (i.e. non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase, GAPDH). Transcripts of ALDH21A1 accumulate in response to desiccation, ABA, UV, and NaCl. The molecular study suggests that ALDH21A1 plays an important role in the detoxification of aldehydes generated in response to desiccation and salt stress; its expression could represent a unique stress tolerance mechanism (Chen et al., 2002b).

Stress-inducible ALDHs

In order to detoxify the cell during stress conditions, the level of metabolic aldehydes and their intermediates must be strictly regulated. The specific pathway(s) in which plant-ALDHs act in stress-tolerance is therefore an area of considerable interest. Skibbe et al. (2002) have used computation approaches to identify amino acid residues likely to be responsible for functional differences between mitochondrial and cytosolic ALDHs of *Zea mays* and *Arabidopsis thaliana*. They reported on the mitochondrial plant-ALDHs such as ZmRF2A, ZmRF2B, OsALDH2a, NtALDH2A, AtALDH2b, AtALDH2a, and the cytosolic plant-ALDHs such as OsALDH1a, ZmRF2C, ZmRF2D, AtALDH1A. Some of these enzymes were confirmed to be related to osmotic stress tolerance, dehydration and salt stress tolerance including members of the ALDH10 family from sorghum (Wood et al., 1996).

Much attention has focused on betaine dehydrogenase genes, wich have been isolated from various plant species. They may also have a dual role in stress response: They are involved in the synthesis of the osmolyte glycine betaine (see later) and they are responsible for the detoxification of betaine aldehyde which is toxic at elevated levels. Velasco et al. (1994) have described a protein family of ALDH11 (GapC-Crat) a cytosolic GAPDH from the resurrection plant *Craterostigma plantagineum*. The mRNA and enzymatic activity of GAPDHc increased in response to dehydration and exogenous application of ABA. From a proteomic study of the Arabidopsis seed, a cytosolic GAPDH peptide was identified to be associated to the desiccation process of the seed, implicating the importance of these enzymes as a conserved biochemical feature for desiccation tolerance (Gallardo et al., 2001). Furthermore, characterization of cDNAs encoding the GAPDH from a desert halophyte *Atriplex nummularia* L. has

been shown to play a crucial role in osmotic stress tolerance (Niu et al., 1994). Wood et al. (1999) have used expressed sequence tags (EST) analysis to discover genes that control vegetative desiccation tolerance in the moss *Tortula ruralis* and characterized several cDNAs at the transcriptional level including ALDH7B6 (Chen et al., 2002a). Table 1 summarizes the different ALDH genes described from plants and their involvement in environmental stress responses.

Over-expression of glycine betaine and proline

Several reports show that plants use various strategies ranging from stomatal closure, slow leaf growth, changes in root morphology and physiology, osmotic adjustment to phenotypic readjustment to cope with stress conditions (Smirnoff, 1998; Pastori and Foyer 2002). Osmotic adjustment is an effective mechanism used by plants in such conditions. Compatible solutes known as osmoprotectants such as glycine betaine and proline accumulate in the cytoplasm of stressed plants and mediate the osmotic adjustment leading to turgor maintenance in plant tissues. Glycine betaine is synthesized through oxidation of choline. This is a two-step reaction where choline is oxidized by choline monooxygenase (CMO) to betaine aldehyde, which is converted to glycine betaine by BADH. Therefore, up-regulation of plant-BADH genes and production of BADH protein during stress is among the target pathways to acquire stress tolerance. Two *Sorghum bicolor* cDNA clones BADH1 and BADH15, putatively encoding betaine aldehyde dehydrogenase, were isolated and characterized by Wood et al. (1996). BADH1 and BADH15 mRNA were both induced under water deficit and their expression coincided with the accumulation of glycine betaine. The accumulation of this compatible solute significantly contributed to an increased osmotic potential and allowed a maximal osmotic adjustment of 0.405 MPa (Wood et al., 1996). Glycine betaine biosynthesis occurs in the chloroplast (Hanson et al., 1985) but BADH is encoded by a nuclear gene (Weretilnyk and Hanson, 1988), and the enzyme is localized in the chloroplast (Weigel et al., 1986). Rathinasabapathi et al. (1994) demonstrated that transgenic tobacco plants expressing either spinach or sugar beet BADH produce a chloroplastic BADH indicating the correct compartmentalization of the process. The engineering of BADH genes has been used to produce transgenic plants which exhibit stress tolerance. Table 2 summarizes examples of improved stress tolerance obtained through overexpressing ALDH genes.

Many plants accumulate also free proline in response to osmotic stress (Delauney and Verma, 1993). Proline may serve as a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989), via reducing the acidity of the cell (Venekamp et al., 1989), and it may function as osmoprotectant at the same time (Kishor et al., 1995). The biosynthetic pathway of proline has been well characterized in *Escherichia coli*. Glutamate is phosphorylated to γ-glutamyl phosphate by gama-glutamyl kinase (γ-GK), which

Table 2. Improved stress tolerance in transgenic plants carrying ALDH cDNA constructs

cDNA used as transgene	Transgenic host plants	Effects observed in transgenic plants	References
Cowpea P5CS	*Tobacco*	Increased proline synthesis and improved tolerance to osmotic stress.	Kishor et al., 1995
Soybean P5CS	*Tobacco*	Increased proline accumulation and improved salt stress tolerance.	Szoke et al., 1995
Spinach BADH	*Tobacco*	Accumulation of glycine betaine.	Rathinasapathi et al., 1994
Beet BADH	*Tobacco*	Enhanced dehydrogenase activity towards 3-dimethylsulfoniopropionaldehyde and two other aldehydes.	Trossat et al., 1997
Vigna aconitifolia P5CF129	*Tobacco*	Increased proline accumulation and improved tolerance to osmotic and oxidative stress.	Hong et al., 2000
Abet A (Choline dehydrogenase)	*Tobacco*	Glycine betaine synthesis and enhanced tolerance to chilling and salt stress.	Holmstrom et al., 2000
BADH-1	*Tobacco*	Glycine betaine synthesis and maintenance of osmotic potential.	Moghaieb et al., 2000
Atriplex hortensis BALDH	*Rice*	Accumulation of glycine betaine and improved growth and salinity tolerance.	Guo et al., 1997
Bet A	*Rice*	Glycine betaine synthesis and improved tolerance to drought and salt.	Takabe et al., 1998
p5cs	*Rice*	Increased biomass production under drought and salinity stress.	Zhu et al., 1998
Anti-ProDH	*Arabidopsis*	Suppression of proline degradation and improved tolerance to freezing and salinity.	Nanjo et al., 1999
CodA (Choline oxidase)	*Arabidopsis*	Synthesis of glycine betaine and improved tolerance to chilling and salt stress.	Hayashi et al., 1997
codA	*Arabidopsis*	Increased tolerance to salt and cold stress.	Huang et al., 2000
codA	*Arabidopsis*	Accumulation of glycine betaine and improved tolerance to salt stress.	Hayashi et al., 1998

is encoded by the proB gene. This is then reduced to glutamic-γ-semialdehyde (GSA) by GSA dehydrogenase (encoded by proA gene). GSA forms spontaneously delta 1-pirroline-5-carboxylate (P5C), which is reduced to proline by delta 1-pirroline-5-carboxylate reductase (P5CR) encoded by the proC gene. Transgenic tobacco overexpressing soybean P5CR shows improved salt tolerance (Szoke et al., 1992). The osmotic potentials of leaf sap from transgenic plants were less decreased under water stress conditions compared to those of control plants. Overexpression of proline also enhanced root biomass and flower development in transgenic plants under drought-stress conditions (Kishor et al., 1995). Examples described here associate the plant-ALDH protein family with strategies leading to stress tolerance (Yancey et al., 1982).

Various conventional research strategies have been used to improve plant tolerance to water stress. Among the most used are the selection of species, which thrive well under water deficit (Nageshawara Rao and Nigam, 2001), and screening for genotypes for deep root systems. Plants with improved water-stress tolerance have been obtained through these strategies. However, they suffer from a major draw back, namely the time it takes to breed for these lines. In contrast, plant biotechnology offers new ways to improve plant-stress tolerance within a shorter time increasing thereby the number of trials. One of the ways to engineer plants with improved water stress tolerance has been the overexpression of genes leading to production of osmoprotectants. It is now possible to improve plant tolerance to various abiotic stresses since cDNAs for both enzymes of glycine betaine synthesis have been cloned from Chenopodiaceae (McCue and Hanson, 1992; Rathinasabapathi et al., 1997). The enzyme mediating the last step of glycine betaine synthesis (BADH) is an NAD-dependent dehydrogenase and also known from Amaranthaceae and Gramineae (Ishitani et al., 1993; Valenzuela-Soto and Munoz-Clares, 1994). Moreover, pairs of homozygous glycine betaine (Bet1/Bet1) lines of *Zea mays* L. exhibited less shoot growth inhibition under salinized conditions in comparison to their near-isogenic glycine betaine deficient bet1/bet1 sister lines (Saneoka et al., 1995). This growth differences were associated with significantly higher leaf relative water content, higher rate of carbon assimilation and greater turgor maintenance under salt stress. This suggests that a single gene transfer conferring glycine betaine accumulation could play a crucial role in osmotic adjustment and improves plant tolerance to water and salt stress.

Conclusion and perspectives

Combinations of tools and approaches have offered unpredicted opportunities to generate transgenic plants with improved stress-tolerance. However, creativity, perseverance, and the use of simple organisms are still needed in order to have a breakthrough in coping with everlasting environmental challenges. Studies carried out on *Arabidopsis thaliana* have made a major contribution to the current understanding of plant-

ALDH functions and their crucial role in plant responses to environmental stresses (Gallardo et al., 2001; Skibbe et al., 2002). The identification and molecular characterization of new plant-ALDH genes could have a potential to improve stress tolerance. Biotechnology and traditional breeding can be used more effectively to produce stress resistant plants once we understand the molecular mechanisms that govern stress tolerance.

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