Alcohol dehydrogenase in plant response to drought

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1. Introduction

Plant growth and productivity is adversely affected by nature’s wrath in the form of various abiotic and biotic stress factors (e. g. salinity, low temperature, drought, and flooding heat, oxidative stress and heavy metal toxicity). All these stress factors are a menace for plants and prevent them from reaching their full genetic potential and limit the crop productivity worldwide. Abiotic stress is the principal cause of crop failure, decrease average yields for most major crops by more than 50% (Bray, 2000) and causes losses worth hundreds of million dollars each year. In fact these stresses, threaten the sustainability of agricultural industry (Shilpi, 2005).

Environmental degradation and climate change have become severe global problems because of the explosive population increases and industrialization in developing countries. To solve this problem, one of the keys is plant biotechnology based on physiology of crop, plant biochemistry, genomics and transgenic technology. This is becoming more and more important for molecular breeding of crops that can tolerate droughts. For this technology, we need to understand plant responses to drought stress at the molecular level.

For agricultural and environmental sustainability, it is important to breed or genetically engineer crops with improved stress tolerance. The identification of key genes and that gene can be used directly for engineering transgenic crops with improved drought tolerance. Although a number of candidate genes have been identified in recent years, only very few have been tested in functional assays for a beneficial effect on drought tolerance. In order to

assess gene function directly in plant suffering from abiotic stress caused by the drought, proved to be useful. Analysing the functions of these genes is critical for understanding of the molecular mechanisms governing plant stress response and tolerance, ultimately leading to enhancement of stress tolerance in crops through genetic manipulation. In this study, this will be used for overexpression of genes as well as for induced gene silencing, by using GATEWAY technology. A comprehensive investigation of Adh and Pdc induction and the determination of ethanol production during stress treatments would provide valuable information on how ethanol involved in the response to limited water condition.

2. Literature review

2. 1. What is stress?

Stress in physical terms is defined as mechanical force per unit area applied to an object. In response to the applied stress, an object undergoes a change in the dimension. Biological term is difficult to define in the plant stress. A biological condition, which may be stress for one plant may be optimum for another plant. The most practical definition of a biological stress is an adverse force or a condition, which inhibits the normal functioning and well being of a biological system such as plants (Jones et al., 1989)

2. 2. Stress signalling pathways

The stress is first perceived by the receptors present on the membrane of the plant cells , the signal is then transduced downstream and this results in the generation of second messengers including calcium, reactive oxygen species (ROS) and inositol phosphates. These second messengers, further modulate the intracellular calcium level. This Ca2+ level is sensed by

calcium binding proteins, Ca2+ sensors. These sensory proteins then interact with their respective interacting partners often initiating a phosphorylation cascade and target the major stress responsive genes or the transcription factors controlling these genes.

The products of these stress genes ultimately lead to plant adaptation and help the plant to survive the unfavourable conditions. Thus, plant responds to stresses as individual cells and synergistically as a whole organism. Stress induced changes in gene expression in turn may participate in the generation of hormones like ABA, salicylic acid and ethylene. The various stress responsive genes can be broadly categorized as early and late induced genes. Early genes are induced within minutes of stress signal perception and often express transiently. In contrast, most of the other genes, which are activated by stress more slowly, i. e. after hours of stress perception are included in the late induced category. These genes include the major stress responsive genes such as RD (responsive to dehydration)/ KIN (cold induced)/COR (cold responsive), which encodes and modulate the LEA-like proteins (late embryogenesis abundant), antioxidants, membrane stabilizing proteins and synthesis of osmolytes.

2. 3. Drought stress

Among all abiotic stresses, drought is one of the most serious problems for sustainable agriculture worldwide. The adverse effect of drought stress is reductions in yield as reported in crops such as rice (Oryza sativa) (Brevedan and Egli, 2003), wheat (Triticum aestivum) (Cabuslay et al., 2002), soybean (Glycine max) (Kirigwi et al., 2004), and chickpea (Cicer aerietum) (Khanna-Chopra and Khanna-Chopra, 2004).

The adaptive responses to drought must be coordinated at the molecular, cellular, and whole-plant levels. These conditions induce dehydration of plant cells, which may trigger physiological, biochemical and molecular responses against such stresses (Shinozaki and Yamaguchi, 1996). Water deficit is a complex of responses, which depends upon severity and duration of the stress, plant genotype, developmental stage, and environmental factors providing the stress.

Yield losses due to drought are highly variable in nature depending on the stress timing, intensity, and duration. Although, different plant species have variable thresholds for stress tolerance, and some of them can successfully tolerate severe stresses and still complete their life cycles, most cultivated crop plant species are highly sensitive and either die or suffer from productivity loss after they are exposed to long periods of stress. It has been estimated that two-thirds of the yield potential of major crops are routinely lost due to unfavourable growing environments (Shilpi, 2005).

Plants have evolved a number of strategies to severe drought. These include escape strategies such as avoidance (flowering, deep rooting, enhanced water uptake efficiency, or reduced water loss) as well as tolerance mechanisms. Reduced shoot growth and increased root development could result in increased water absorption and reduced transpiration, thereby maintaining plant tissue water status. In addition to such avoidance mechanisms, plant responses to water shortages can involve changes in biochemical pathways and expression of genes encoding proteins that contribute to drought adaptation. The proteins could be enzymes involved in the synthesis of osmolytes, antioxidants, or hormones such as ABA and

others. Such changes can bring about drought tolerance, whereby plants continue to function at the low water potentials caused by water deficit (Hall, 1993). A central response to water deficit is often increased synthesis of ABA, which in turn induces a range of developmental (avoidance) and physiological or biochemical (tolerance) mechanisms. There is an ongoing debate as to whether the exploitation of avoidance or tolerance mechanisms should be the focus of plant breeding programmes. However, it appears likely that the exploitation of tolerance mechanisms may be more promising for the stabilization of crop yield under severe drought conditions (Araus et al, 2002).

An assortment of genes with diverse functions are induced or repressed by these drought stresses (Bartels and Sunkar, 2005; Yamaguchi and Shinozaki, 2005). Drought tolerance has been shown to be a highly complex trait, regulated expression of multiple genes that may be induced during drought stress and thus more difficult to control and engineer. Plant engineering strategies for abiotic stress tolerance rely on the expression of genes that are involved in signaling and regulatory pathways (Seki and Shinozaki, 2003) or genes that encode proteins conferring stress tolerance (Wang, 2004) or enzymes present in pathways leading to the synthesis of functional and structural metabolites. Current efforts to improve plant stress tolerance by genetic transformation have resulted in several important achievements; however, the genetically complex mechanisms of abiotic stress tolerance make the task extremely difficult.

2. 3. 1 Physiological and biochemical responses of drought

Physiological and biochemical changes at the cellular level that are associated with drought stress include turgor loss, changes in membrane fluidity and composition, changes in solute concentration, and protein and protein-lipid interactions (Chaves et al, 2003) .

Other physiological effects of drought on plants are the reduction in vegetative growth, in particular shoot growth. Leaf growth is generally more sensitive than the root growth. Reduced leaf expansion is beneficial to plants under water deficit condition, as less leaf area is exposed resulting in reduced transpiration. Many mature plants, for example cotton subjected to drought respond by accelerating senescence and abscission of the older leaves. This process is also known as leaf area adjustment. Regarding root, the relative root growth may undergo enhancement, which facilitates the capacity of the root system to extract more water from deeper soil layers.

Plant tissues can maintain turgor during drought by avoiding dehydration, tolerating dehydration or both (Kramer, 1995). These forms of stress resistance are controlled by developmental and morphological traits such as root thickness, the ability of roots to penetrate compacted soil layers, and root depth and mass (Pathan, 2004). By contrast, adaptive traits, such as osmotic adjustment and dehydration tolerance, arise in response to water deficit . Reduction of photosynthetic activity, accumulation of organic acids and osmolytes, and changes in carbohydrate metabolism, are typical physiological and biochemical responses to stress.

Synthesis of osmoprotectants, osmolytes or compatible solutes is one of the mechanisms of adaptation to water deficit. These molecules, which act as osmotic balancing agents, are accumulated in plant cells in response to drought stress and are subsequently degraded after stress relief (Tabaeizadeh , 1998).

2. 3. 2 Molecular responses

Studies on the molecular responses to water deficit have identified multiple changes in gene expression. Functions for many of these genè products have been predicted from the deduced amino acid sequence of the genes. Genes expressed during stress are anticipated to promote cellular tolerance of dehydration through protective functions in the cytoplasm, alteration of cellular water potentia1 to promote water uptake, control of ion accumulation, and further regulation of gene expression.

Expression of a gene during stress does not guarantee that a gene product promotes the ability of the plant to survive stress. The expression of some genes may result from injury or damage that occurred during stress. Other genes may be induced, but their expression does not alter stress tolerance. Yet others are required for stress tolerance and the accumulation of these gene products is an adaptive response.

Complex regulatory and signaling processes, most of which are not understood, control the expression of genes during water deficit. In addition to induction by stress, the expression of water-deficit-associated genes is controlled with respect to tissue, organ, and developmental stage and may be expressed independently of the stress conditions. The regulation of

specific processes will also depend upon the experimental conditions of stress application. Stress conditions that are applied in the laboratory may not accurately represent those that occur in the field. Frequently, laboratory stresses are rapid and severe, whereas stress in the field often develops over an extended period of time (Radin, 1993). These differences must also be evaluated when studying the adaptive value of certain responses. The function of the gene products and the mechanisms of gene expression are intertwined, and both must be understood to fully comprehend the molecular response to water deficit.

2. 4. Function of water-stress inducible genes

Genes induced during water-stress conditions are thought to function not only in protecting cells from water deficit by the production of important metabolic proteins but also in the regulation of genes for signal transduction in the water-stress response .

Thus, these gene products are classified into two groups. The first group includes proteins that probably function in stress tolerance: water channel proteins involved in the movement of water through membranes, the enzymes required for the biosynthesis of various osmoprotectants (sugars, Pro, and Gly-betaine), proteins that may protect macromolecules and membranes (LEA protein, osmotin, antifreeze protein, chaperon, and mRNA binding proteins), proteases for protein turn over (thiol proteases, Clp protease, and ubiquitin), the detoxification enzymes (glutathione S-transferase, soluble epoxide hydrolase, catalase, superoxide dismutase, and ascorbate peroxidase). Some of the stress-inducible genes that encode proteins, such as a key enzyme for Pro biosynthesis, were over expressed in

transgenic plants to produce a stress tolerant phenotype of the plants; this indicates that the gene products really function in stress tolerance (Shinozaki , 1996).

The second group contains protein factors involved in further regulation of signal transduction and gene expression that probably function in stress response: Most of the regulatory proteins are involved in signal transduction. Now it becomes more important to elucidate the role of these regulatory proteins for further understanding of plant responses to water deficit. Many transcription factor genes were stress inducible, and 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 Arabidopsis (Pathan. 2004),

2. 5. Model plant for studying the drought tolerant

Arabidopsis thaliana is a small weed in the mustard family. It has been a convenient for studies in classical genetics for over forty years (Redei, 1975). This flowering plant also has a genome size and genomic organization that recommend it for certain experiments in molecular genetics and it is coming to be widely used as a model organism in plant molecular genetics, development, physiology, and biochemistry. Arabidopsis thaliana provides an excellent experimental plant system for molecular genetics because of its remarkably small genome size and short life cycle. Arabidopsis thaliana, a genetic model plant, has been extensively used for unravelling the molecular basis of stress tolerance. Arabidopsis also proved to be extremely important

for assessing functions for individual stress associated genes due to the availability of knock-out mutants and its amenability for genetic transformation. It has been collected or reported in many different regions and climates, ranging from high elevations in the tropics to the cold climate of northern Scandinavia and including locations in Europe, Asia, Africa, Australia, and North America (Kirchheim, 1981).

Arabidopsis has the smallest known genome among the higher plants. The reasons for a small genome include little repetitive DNA and, in some cases, simpler gene families. Leutwiler et al. (1984) reported that the haploid genome from Arabidopsis (n = 5 chromosomes) contains only roughly 70, 000 kilobase pairs (kb). The contrast of the Arabidopsis genome with that of other plants frequently used in molecular genetic work is striking: tobacco, for example, has a haploid nuclear genome of 1, 600, 000 kb; the pea haploid genome is 4, 500, 000 kb; and the wheat haploid genome is 5, 900, 000 kb . The significance of this small DNA content for molecular genetics is that a genomic library of Arabidopsis chromosomal fragments is easy to make, and simple and economical to screen. It is thus rapid and inexpensive to repeatedly screen Arabidopsis genomic libraries. In addition to its remarkably low content of nuclear DNA, Arabidopsis has a genomic organization that makes it uniquely suited to certain types of molecular cloning experiments.

All of the properties of the plant —small, short generation time, high seed set, ease of growth, self- or cross-fertilization at will–make Arabidopsis a convenient subject for studies in classical genetics.

2. 6. Drought related gene

Alcohol dehydrogenase and pyruvate decarboxylase are enzyme whose activity has been observed in numerous higher plants including Arabidopsis, maize, pearl millet, sunflower, wheat, and pea (Gottlieb, 1982). In a number of plants, different ADH genes are expressed in various organs, at specific times during development, or in re-sponse to environmental signals. High levels of ADH activity are found in dry seeds and in anaerobically treated seeds (Freeling, 1973. Banuett-Bourrillon . 1979), roots (Freeling . 1973), and shoots (App, 1958).

During periods of anaerobic stress, the enzyme is presumably required by plants for NADH metabolism, via reduction of acetaldehyde to ethanol. With respect to secondary metabolites, ADH is involved in the inter conversion of volatile compounds such as aldehydes and alcohols (Bicsak et al., 1982; Molina et al., 1986; Longhurst et al., 1990).

The ethanolic fermentation pathway branches off the main glycolytic pathway at pyruvate. In the first step, pyruvate is the substrate of pyruvate decarboxylase (PDC), yielding CO2 and acetaldehyde. Subsequently, acetaldehyde is reduced to ethanol with the concomitant oxidation of NADH to NAD+ by alcohol dehydrogenase (ADH).

Although PDC and ADH gene induction has been demonstrated, ethanol and acetaldehyde production as a result of stress treatment has only been reported for red pine (Pinus resinosa) and birch (Betula spp.) seedlings exposed to sulfur dioxide, water deficiency, freezing, and ozone(Kimmerer and Kozolowski. 1982).

Many plants contain more than one ADH gene (Gottlieb, 1982), resulting in the expression of different ADH proteins (i. e. ADH isozymes, often designated ADH 1, ADH2, etc.). The most extensive study of maize Adh genes, AdhI and Adh2, have been cloned and sequenced. The coding sequences of these genes are 82% homologous, interrupted by nine identically positioned introns that differ in sequence and length.

The expression of the Arabidopsis Adh gene (Chang and Meyerowitz, 1986; Dolferus et al., 1990) has many features in common with maize Adhl gene (Walker et al., 1987). The two genes have comparable developmental expression pattens, and both have tissue-specific responses to hypoxic stress. In both maize and Arabidopsis, the gene is expressed in seeds, roots, and pollen grains, whereas green aerial plant parts are devoid of detectable levels of ADH activity. In both species, hypoxic induction of the gene occurs in cells of the root system (reviewed by Freeling and Bennett, 1985; Dolferus and Jacobs, 1991; Okimoto et al., 1980;). ADH is induced anaerobically in Arabidopsis (Dolferus, 1985) as in maize. ADH is also induced in both maize root and Arabidopsis callus by the synthetic auxin 2, 4-dichlorophenoxyacetic acid (Dolferus, 1985. Feeling, 1973).

Several approaches have been undertaken to assess the functional role of Adh in development, stress response, and metabolite synthesis. The expression of the alcohol dehydrogenase (Adh) gene is known to be regulated developmentally and to be induced by environmental stresses (Christie et al., 1991; Bucher et al., 1995). Alcohol dehydrogenase (ADH) plays a key enzymatic function in the response to anaerobic conditions in plants (Sachs, Subbaiah, and Saab 1996). A new and exciting aspect of

ethanolic fermentation is the suggested involvement in stress signaling and response to environmental stresses other than low oxygen (Tadege et al., 1999). Furthermore, specific analysis of the ADH gene from rice (Oryza sativa), maize, and Arabidopsis showed ADH to be induced by cold (Christie et al., 1991), wounding (Kato-Noguchi, 2001), dehydration (Dolferus et al., 1994), and the phytohormone abscisic acid (ABA; de Bruxelles et al., 1996), in line with the observation from the micro-array experiments.

In Arabidopsis thaliana, Adh overexpression improved the tolerance of hairy roots to low oxygen conditions and was effective in improving root growth (Dennis et al., 2000; Shiao et al., 2002). However, it had no effect on flooding survival (Ismond et al., 2003). Adh over expression in tomato has been shown to modify the balance between Câ,†, Adh overexpression in tomato aldehydes and alcohols in ripe fruits (Speirs et al., 1998). Grapevine plants overexpressing Adh displayed a lower sucrose content, a higher degree of polymerization of proanthocyanidins, and a generally increased content of volatile compounds, mainly in carotenoid- and shikimate-derived volatiles (Catherine et al., 2006).