Osmoprotection in Sugarcane under Water Deficit Conditions

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ABSTRACT

Drought has become a limiting factor for expansion of agricultural areas. Plant stress caused by water deficit is a major abiotic agent that affects many crops throughout the world, causing decrease in productivity and consequently economic losses. Development of more tolerant cultivars seems to be the right way to overcome adverse environmental conditions and increase productivity. Plant adaptive response to unfavorable conditions occurs via distinct mechanisms such as salinity, drought, or high temperatures. This review covers some of the biochemical and genetic mechanisms which are related to plant tolerance and how they may interact to induce plant tolerance to drought. The role of signaling molecules, osmoregulators and Reactive Oxygen Species (ROS) in plant response mechanisms are discussed. In addition modifications at the transcriptional level are pointed out with the characterization of sugarcane gene expression profile under water stress conditions. Understanding how sugarcane gene expression is regulated and how its biochemical machinery is mobilized in response to drought will promote the development of tolerant cultivars. Based on the overall results already reported and in our findings on the differential expression of genes related to water stress cell response a coordinated mechanism of tolerance and sensitivity is proposed.

Keywords: abiotic stress, drought, gene expression, genetic improvement

INTRODUCTION

In their native habitats, plants frequently grow under challenging conditions such as drought, salinity, frost, high temperature, floods or high luminosity. These conditions are collectively known as abiotic stresses and each one of them may delay growth and development, reduce plant productivity and, in more extreme circumstances, cause plant death (Jiang and Zhang 2002; Osturk et al. 2002; Chen and Murata 2002; Xiong et al. 2002; Rabbani et al. 2004; Garcia et al. 2007). Under abiotic stress, changes in gene expression such as induction and/or repression of genes occur, and these changes may be directly regulated by stress conditions or may result from secondary stresses and/or as a response to injuries to metabolic and cellular functions (Bray 2002). In addition, genotypes that differ in tolerance to water deficit should present qualitative and quantitative differences in gene expression. Thus, a specific physiological response to water deficit results from a combination of previous molecular events, activated by the perception of the stress signaling molecules. It is necessary, therefore, to understand how these molecular events are activated/deactivated and how they interact. Crop plants can be genetically modified with the introduction of genes which are able to confer tolerance, thus maintaining plant productivity under adverse conditions (Taylor 1996; Nepomuceno et al. 2001; Bray 2002; Rabbani et al. 2003; Rodrigues et al. 2009; Zingaretti et al. 2011). This explains the importance of studying different genotypes of a species, to better understand gene profiles involved in the responses to stress.

COMPATIBLE SOLUTES

In order to survive plants have presented, throughout their evolution, a wide range of strategies that have allowed their survival when faced with various abiotic stresses. One of such mechanism is the accumulation of compatible solutes, which protect cell structure against damage induced by dehydration and oxidation. For this reason the term “compatible solute”, sometimes used to describe the osmolytes is not completely appropriate, since these compounds are not merely compatible (harmless), but also protective, they can also act as free radical scavengers or chemical chaperones by directly stabilizing membranes and/or proteins. It is important to denote that osmolyte synthesis may have additional physiological functions, such as for example, helping redox control by consuming equivalent oxidizer. This consumption may be particularly beneficial during

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dehydration due to disturbances in the electron transport chain and an increase in the formation of reactive oxygen species from equivalent cell reducers induced by stress (Serrano and Montesinos 2003). The accumulated compatible solutes differ among plant species and may include betaines and related compounds, sugars (mannitol, sorbitol and trehalose) and amino acids such as proline and hydroxyproline (Chen and Murata 2002).

Proline builds up in many plant species under a wide range of biotic and abiotic stresses (Hare and Cress 1997; Xiong and Zhu 2002; Abraham et al. 2003; Deuschle et al. 2004; Clausen 2005). Its accumulation is observed under limited water conditions (drought, salinity or low temperature) and during desiccation processes, such as pollen maturation (Deuschle et al. 2004). In sugarcane the free proline content can go from 0.4 to 1.6 μg/g FW under water restriction conditions (Guimarães et al. 2008). Like other compatible solutes, proline presents low molecular weight, no charge at neutral pH, is highly soluble and in high concentrations has little or no disturbing effect in solvent-macromolecular interactions (Chen and Murata 2002). Proline also acts as resource for carbon, nitrogen and energy during stress recovery, being promptly oxidized to glutamate (Raymond and Smirnoff 2002). Among many compatible solutes, this amino acid accumulated in plasma that has proven to protect plants against damage induced by singlet oxygen and free radicals (Kishor et al. 2005). Since proline can eliminate singlet oxygen and OH· radicals, it plays an important role to stabilize protein structures, DNA, as well as membranes and subcellular structures against denaturation (Iyer and Caplan 1998; Maggio 2005). Investigating the activity to eliminate hydroxyl radicals of different compounds (mannitol, myo-inositol, sorbitol and proline), Kishor et al. (2005) showed that proline represents the most efficient hydroxyl radical scavenger. Proline also appears to operate on pH regulation, as a relief mechanism for cytosolic acidity, a condition frequently associated with stress. According to Hare and Cress (1997), a decrease in intracellular pH has been appointed as a factor that can elicit proline build up in plants and, the removal of H+ due to proline synthase prevented a depression in soybean plant respiration under saline or drought stresses. Proline can be considered an important component in the molecular signaling to stress cascade and a main constituent of proteins in plant cell walls (Nepomuceno et al. 2001; Deuschle et al. 2004).

To understand the mechanisms by which plants perceive environmental signals and transmit them to cells in order to activate adaptive responses, is very important for the development of cultivars. It is a process that transduction pathway starts with signal perception, followed by the generation of secondary messengers like Ca2+ (Bethke et al. 1995). The modulation of Ca2+ levels, frequently initiate a protein phosphorylation cascade, which finally reaches target-proteins directly involved in cell protection of transduction factors controlling specific groups of stress-regulated genes (Xiong et al. 2002). One of the first responses to stress is the change of turgor pressure, dry mass and by proton gradient, which is very sensitive to plant signals induced by stress could involve a reduction in turgor pressure, changes to phytohormone levels, and in free cytosolic calcium ([Ca2+]c). An association between abscisic acid (ABA) and proline buildup seems to be non-existent in tomatoes, though it has been suggested for some investigated plant species (Clausen 2005). Shah et al. (2001) studied the effects of calcium on proline buildup in suspension-cultured rice cells stressed by NaCl and the results showed that proline concentrations in non-stressed cells regardless of calcium levels, was similar. However, regarding NaCl-stressed cells, a large increase in proline concentration was observed in cells supplemented with calcium compared to a small increase in proline levels in cells grown under low calcium levels. The authors conclude that calcium supplementation seems to have a role in proline buildup of suspensions-cultured rice cells stressed by NaCl, mainly at the level of mRNA translation in relation to DNA transcription.

Ca2+ may be a key second messenger signal transducer of various stress stimuli, and has been related to protection against stress for stabilizing membranes and reducing oxidative damages. According to Nayyar (2003), the increase in cytosolic Ca2+ induced by stress could in fact be inducing proline biosynthesis, considering that in Arabidopsis an inhibition of Δ-pyrroline-5-carboxylate synthase (P5CS) enzyme transcription occurred when Ca2+ channel blocker was used. In maize suspension cultured cells the cytosolic calcium content increased from 25 to 150 μM, under O2 deprivation (Subaiah et al. 1994).

The proline increase under stress conditions may be triggered by induction or activation of enzymes involved in its biosynthesis; by a decrease in proline oxidation to glutamate; a decrease in its use for protein synthesis, or even by an increase in proline synthesis. In plants, the expression of genes that code for key-enzymes of proline biosynthesis, through amino acid glutamate as precursor P5CS (EC:2.7.2.11/1.2.1.41) Δ-pyrroline-5-carboxylate-synthetase and P5CR (EC:1.5.1.12) Δ-pyrroline-5-carboxylate-reductase and for proline oxidation (PDH, EC 1.5.99.8, proline dehydrogenase) is modulated by osmotic and salinity stresses and precedes the increase or decrease in proline concentrations on plant tissue (Clausen 2005). Proline oxidation to glutamate is restricted to mitochondria and is catalyzed by the action, in sequence, of proline dehydrogenase (PDH), and (P5C, EC 1.5.1.12) dehydrogenase (P5CDH) enzymes (Hare and Cress 1997).

In plants, proline can be synthesized through different pathways, one from glutamate and the other from ornithine. The Δ-pyrroline-5-carboxylate-synthetase is a bi-functional enzyme in plants. Its first activity, γ-glutamyl kinase, catalyzes ATP-dependent phosphorylation of L-glutamate and the resultant γ-glutamyl phosphate is then reduced to glutamate-semialdehyde (GSA) by GSA dehydrogenase NADPH-dependent (GSA redtuse). This intermediary is spontaneously converted to pyrroline-5-carboxylate, which is reduced to proline by Δ-pyrroline-5-carboxylate reductase (P5CR), also NDHADP-dependent. All these reactions for the synthesis of proline, with glutamate as precursor, however, when a signal is received that proline synthesis is to be triggered by induction or activation of enzymes involved in its biosynthesis, through amino acid glutamate as precursor P5CS (EC:2.7.2.11/1.2.1.41) Δ-pyrroline-5-carboxylate-synthetase and P5CR (EC:1.5.1.12) Δ-pyrroline-5-carboxylate-reductase and for proline oxidation (PDH, EC 1.5.99.8, proline dehydrogenase) is modulated by osmotic and salinity stresses and precedes the increase or decrease in proline concentrations on plant tissue (Clausen 2005). Proline oxidation to glutamate is restricted to mitochondria and is catalyzed by the action, in sequence, of proline dehydrogenase (PDH), and (P5C, EC 1.5.1.12) dehydrogenase (P5CDH) enzymes (Hare and Cress 1997).

Genetic regulation of the enzymes from proline metabolism is sensitive to environmental conditions that affect free proline concentrations. In Arabidopsis, the mRNA for Δ-pyrroline-5-carboxylate-synthetase (P5CS) quickly accumulates as a result of desiccation, NaCl stress and ABA treatment. Induction correlates well with free proline concentrations, which increases from 4 to 8 times in 24 h after desiccation or ABA treatment. This correlation is reinforced through the observation that both the mRNA and free proline decrease coordinately when plants are rehydrated. This regulation in plants occurs on enzyme level as well as through changes in gene expression. The Δ-pyrroline-5-carboxylate-synthetase enzyme (P5CS) seems to be the limiting factor in plants. Consistent with the theory that Δ-pyrroline-5-carboxylate-reductase (P5CR) is not the limiting factor; its overexpression had no effect on free proline in
transgenic tobacco plants (Buchanan et al. 2000). Regarding proline catabolism, the first step is its oxidation into $P$SC by mitochondrial enzyme proline dehydrogenase (PDH). In plants, this enzyme is linked to the electron transport system and then couples proline degradation with ATP formation. PDH activity is reduced in isolated mitochondria of plants under water stress, suggesting that the proline catabolic pathway may be repressed in mitochondria under these circumstances. This results in an increase in the cytosolic levels of proline (Taylor 1996).

Prolyl hydroxylase (PH, EC1.14.11.12) enzyme is a plant dioxygenase that converts proline into hydroxyproline. Molecules containing hydroxyproline can be found in the cell walls of all plants. Many are differentially regulated during growth and in response to stress, for example hydroxyproline, an important component of extensin, a protein found in plant cell walls. Hydroxyproline synthesis from proline differs from all other amino acids synthesis because the reaction occurs after proline has been incorporated in the protein and is, therefore, a post-translational modifying reaction. Prolyl hydroxylase is located in the endoplasmic reticulum, which suggests that the most of the proteins containing hydroxyproline is found in the secretory pathway (Buchanan et al. 2000). This enzyme requires L-ascorbate, a cofactor to increase its activity (Daves et al. 2000). According to Oda et al. (2005), it has been reported in literature that the hydroxyproline amino acid, as well as proline, act as osmoprotectants in plants under water and salinity stress.

The $\gamma$-aminobutyric acid (GABA), a four-carbon non-protein amino acid, is an important component of the free amino acids pool. GABA is highly water-soluble, and a structurally flexible molecule that can take different forms in solution, including a cyclic structure similar to proline. The pathway that converts glutamate to succinate through GABA is called “GABA shunt”. The first step is a direct and irreversible decarboxylation of glutamate by glutamate decarboxylase (GAD, EC 4.1.1.15), a cytosolic enzyme. Typically, GABA levels in plant tissues is low, but it increases many times in response to various stimuli, including thermal shock, mechanical stimulation, drought, hypoxia and phytohormones (Shelp et al. 1999), showing that GABA is intensively and rapidly produced as a result of abiotic and biotic stresses. The GABA shunt has been associated with many physiological responses such as cytosolic pH regulation, the carbon influx into the Krebs cycle, nitrogen metabolism, and protection against oxidative stress, osmo-regulation, and cell signaling (Bouché and Fromm 2004).

It has been suggested that GABA synthesis induced by stress is a result of cytosolic acidity and the consequent GAD stimulation. However, it is unlikely that the numerous environmental factors that stimulate GABA accumulation are all mediated by a decrease in the cytosolic pH. It is known that stress factors such as mechanical or cold shock that stimulate GABA levels, increase cytosolic calcium levels which induce $Ca^{2+}$/calmodulin-dependent glutamate decarboxylase (GAD) activity and, therefore, induce GABA synthesis. According to Shelp et al. (1999), studies carried out in different petunia organs showed the presence of differential expression patterns of mRNA and GAD protein, suggesting that GAD activity is regulated on a transcriptional as well as translational level (Shelp et al. 1999).

The Arabidopsis (AtProT2) and tomato (LeProT1) transporters carry GABA as well as other components related to stress such as proline and glycine-betaine. The AtProT2 transporter can be induced by water and salinity stress. The findings have indicated that GABA may have a role as compatible osmolyte, considering that it is also highly water-soluble and has no toxic effect. In high concentrations, GABA presents cryoprotectant properties, stabilizing and protecting isolated thylakoids against frost in the presence of salt, as well as possessing hydroxyl radical elimination activities (Shelp et al. 1999; Bouché and Fromm 2004). According to Shelp et al. (1999), whether GABA has a specific role (as osmolyte or osmoprotectant) under water stress or it is metabolized (to produce proline), remains unknown.

**OXIDATIVE STRESS AND REACTIVE OXYGEN SPECIES (ROS)**

Oxygen is essential to aerobic life existence, but toxic by-products called reactive oxygen species (ROS) such as singlet oxygen ($O_2^*$), superoxide radicals ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ($OH$), are generated in all aerobic cells during normal cellular metabolism (respiration, photosynthesis, photorepiration and beta oxidation of fatty acids) in mitochondria, chloroplasts and peroxisomes (Guan, Zhao and Scandalios 2000; Kwon et al. 2002; Xiong and Zhu 2002; Apel and Hirt 2004; Gill and Tuteja 2010). However, ROS levels increase as a consequence of various environmental injuries to which plants are exposed, such as temperature, oxygen deprivation (Blökhina et al. 2003), high light intensity (Apel and Hirt 2004), water stress (Jiang and Zhang 2002), salinity stress (Hernández et al. 2000), mechanical stress or even pollution.

ROS can react with a variety of biomolecules, altering or blocking their biological functions, causing damage to cell components such as membrane lipids, deactivating enzymes (denaturation), carbohydrates, nucleic acids and to the photosystem II complex (Guan et al. 2000; Arora et al. 2002; Chen and Murata 2002; Blökhina et al. 2003; Apel and Hirt 2004). The injuries caused by ROS are known as oxidative stress and constitute one of the main damage factors in plants exposed to different environmental stresses (Kwon et al. 2002). According to Chen and Polle (2010), ABA, $Ca^{2+}$ and ROS are involved in abiotic stress sensing, like soil salinity, with higher or faster activation of defenses in tolerant than susceptible poplar species. In order to reduce toxic effects of ROS, plants use highly regulated enzymatic and non-enzymatic mechanisms to maintain a balance between the production and destruction of ROS to maintain cell redox homeostasis (Guan et al. 2000; Blökhina et al. 2003; Sarram and Tyagi 2004). The term antioxidant can be considered to describe any compound able to extinguish ROS without undergoing conversion into a harmful radical. Antioxidant enzymes are those that catalyze such reactions or involved in ROS metabolism. Consequently, antioxidants and antioxidant enzymes interrupt cascades of uncontrolled oxidation (Noctor and Foyer 1998). Therefore, plants possess the ability to fight against oxidative stress using ROS-eliminating systems such as superoxide dismutase (SOD, EC1.15.1.1), catalase (CAT, EC1.11.1.6), ascorbate peroxidase (APX, EC1.11.1.1), as well as antioxidant compounds of low molecular weight such as glutathione (GSH), glutatione-S-transferase, phenolic compounds and polyamines. In addition to the ROS-eliminating enzymes, SOD and APX, enzymes such as monodehydroascorbate reductase (MDHAR, EC1.6.5.4), dehydroascorbate reductase (DHAR) and glutathione reductase (GR, EC1.8.1.7), which are necessary for the regeneration of ascorbate and glutathione are also involved.

**SUPEROXIDE DISMUTASE (SOD)**

Superoxide dismutase (SOD) is the first enzyme reported as being able to decompose a free radical. This enzyme catalyzes the superoxide radical as shown in the flowing reaction, $O_2^- + O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$ reaction (Gupta et al. 1993; Netto 2001). In year 2001, Netto analyzed sugarcane ESTs (SUCEST databank) and found 5 isoforms of superoxide dismutase (SOD). According to the author, the high number of isoforms may indicate that the superoxide radical can be very toxic to plants, though this free radical is not very reactive. Moreover, the author suggested that superoxide radical toxicity occurs due to its ability to react with nitric oxide to form peroxynitrite, which is a strong oxidant. Furthermore, superoxide ($O_2^-•$) and hydrogen peroxide ($H_2O_2$), in spite of not being very toxic, in the presence of trace amounts of $Fe^{2+}$ and $Fe^{3+}$ through the Haber-Weiss reaction, forms the hydroxyl radical ($OH$). This radical can
cause damage to chlorophyll, proteins, DNA, lipids and other important macromolecules, thus can cause fatally affect plant growth and development, and finally and hence ultimately affect plant productivity (Sairam and Tyagi 2004).

**CATALASE (CAT)**

The catalase enzyme protects cells from hydrogen peroxide that may be generated from the reaction catalyzed by superoxide dismutase, through the beta-oxidation of fatty acids in peroxisomes or through other processes like the Mehler reaction in chloroplasts, in which $\text{H}_2\text{O}_2$ is generated under normal metabolism; by electron transport in mitochondria and photosrespiration in peroxisomes (Neill et al. 2002). This hemeprotein catalyzes the reaction $2\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}_2\text{O}$ (Gupta et al. 1993; Netto 2001). Plant catalasas derive from a common ancestor gene and can be divided into three distinct groups (CAT1, CAT2, and CAT3). Moreover, similar clusters of catalasas were identified in the sugarcane expressed sequence tags data base (SUCEST) databank (Netto 2001).

**ASCORBATE PEROXIDASE (APX)**

Ascorbate peroxidase (APX) is another key enzyme for the control of hydrogen peroxide concentrations due to its ability to catalyze the decomposition of hydrogen peroxide at the expense of ascorbate (ASC). The sequence of this protein containing heme distinguishes itself from other peroxidases and different APX forms occur in chloroplasts, cytosol, mitochondria, peroxisomes and glyoxysomes.

Catalases (CAT) convert hydrogen peroxide ($\text{H}_2\text{O}_2$) into water and molecular oxygen. These enzymes have extremely high catalytic levels, but low affinity with the substrate, since the reaction requires two $\text{H}_2\text{O}_2$ molecules to activate its site. An alternative way of breaking down $\text{H}_2\text{O}_2$ is through peroxidases, which are found in every cell and have high affinity with $\text{H}_2\text{O}_2$ compared to CAT. Peroxidases, however, require a reducer since they reduce $\text{H}_2\text{O}_2$ to water. In plant cells, the most important reduced substrate for $\text{H}_2\text{O}_2$ detoxification is ascorbate. APX uses two ascorbate molecules to reduce $\text{H}_2\text{O}_2$ to water, with the simultaneous generation of two monodehydroascorbate molecules (MDHA) (Noctor and Foyer 1998). Two enzymes are involved in reduced ASC regeneration, called monodehydro-ascorbate reductase (MDHAR), which uses NADPH directly in recycling ASC and dehydroascorbate reductase (DHAR).

Although the interrelation of water stress, ABA, ROS and the antioxidant defense system had been studied in several plant species remains unclear which signals stimulate the increase of antioxidant enzymes, that are essential to the defense against oxidative stress According to Apel and Hirt (2004), the generation of ROS into cell compartments, such as the mitochondria or chloroplasts results in changes to the nuclear transcripions, indicating which information should be transmitted from these organelles to the nucleus, but the identity of the transmitted signal remains unknown (Gill and Tuteja 2010). There are three possible ways to indicate how ROS could affect gene expressions. ROS sensors could be activated, inducing signal cascades that ultimately culminate gene expression. Another alternative would be that signaling pathways could directly oxidized by ROS. And finally, ROS could change gene expression, affecting and modifying the activity of transcription factors.

According to Netto (2001), plants have developed different systems to confront the toxic effects of reactive oxygen (ROS) and nitrogen (RNS) species. The first antioxidant defense line involves the prevention of ROS formation. The second is formed of antioxidant enzymes and compounds of low molecular weight. Moreover, if the first antioxidant line of defense fails to prevent the formation of reactive species, antioxidant compounds break down reactive species, thus avoiding the generation of oxidative injuries in the biomolecules (Netto 2001). According to Hoekstra (2002), drought and desiccation tolerant cells undergo less oxidative damages than cells that are sensitive to these stresses. This could be the result of an effective decrease in ROS production or of the activation of effective antioxidant systems, or both.

**SUGARCANE RESPONSE TO WATER STRESS**

Sugarcane (Saccharum spp.) belongs to the Poaceae family, and is an important source of sucrose and ethanol in many tropical and sub-tropical countries. Though, it was introduced in Brazil centuries ago only in the last decades has become one of the most important crops in Brazil, currently, the world’s largest sugarcane producer. Sugarcane, among other crops, produces a higher amount of biomass per unit of cultivated area and water availability is very important throughout its different developmental stages (Zingaretti et al. 2011). As water resources in the planet becoming limited, the development of more efficient cultivars for water usage is extremely important. It has become a challenge to better understand sugarcane responses to water deficit and several reports have been published over the last few years on this subject (Papini-Terzi 2005; Rocha et al. 2007; Rodrigues et al. 2009, 2011; Kido et al. 2012) and the molecular approaches concerning sugarcane responses to stress have been conducted by using techniques based on hybridization and the comparison of sensitive and tolerant cultivars. It is well known that gene expression alterations can promote cellular adaptation to water stress. The profile of expressed genes that are characterized in scientific studies suggests the complexity of defense mechanisms as those exhibited by Gossypium (Chaudhary et al. 2009), Saccharum spp. (Kido et al. 2012); Hordeum vulgare L. (Rodriguez-Serrano et al. 2012) and others. Those mechanisms involve a complex network of signaling molecules, genes responsive to stress and regulation by plant hormones, among others that seem to be related with protection against metal toxicity and oxidative stress (Jain et al. 2006).

Rocha et al. (2007), described genes differentially expressed by a sugarcane cultivar sensitive to water deficit. Their results indicated that drought elicited changes mostly in the late experimental phase 72 h and 120 h after the onset of drought. About 88% of drought-responsive genes were detected as differentially expressed exclusively after 72 h.

### Table 1 Comparative gene expression for tolerant and sensitive sugarcane cultivars.

<table>
<thead>
<tr>
<th>Description</th>
<th>Fold change</th>
<th>Mild</th>
<th>Severe</th>
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<tr>
<td>Proline biosynthesis</td>
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<td>putative PSCS</td>
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<td>2.48</td>
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<td>PSCS</td>
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<td>-2.63</td>
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<td>PSCR</td>
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<td>-1.81</td>
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<tr>
<td>putative δ-OAT</td>
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<td>2.03</td>
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<tr>
<td>putative δ-OAT</td>
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<td>-1.44</td>
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<td>Antioxidant defense systems</td>
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<tr>
<td>Mn-SOD</td>
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<td>DHAR</td>
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<td>GABA biosynthesis</td>
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<tr>
<td>putative GAD isozyme</td>
<td>1.44</td>
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<td>-1.19</td>
<td>-1.42</td>
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*Fold changes indicate the level of stress Original data*
and/or 120 h of water deprivation.

Rodrigues et al. (2011), using macroarray technology and a water stress-tolerant cultivar, verified that plant response starts with stress recognition at a cellular level and activation of signal transduction pathways. Genes that encode calcium binding proteins as well as kinase and phosphatase proteins are activated by extracellular stimulus, which induce gene expression changes. Once activated, transcription factors act as DNA-binding proteins that are able to mediate the transcription of key proteins in the stress response mechanism. Methods to increase environmental stimulus perception or to intensify cellular communication could facilitate the anticipation of defense mechanisms (Chinnusamy et al. 2004). In the microarray study, ABA-regulated proteins encoding genes were induced during the entire stress exposure period. Previous studies with Arabidopsis ABA mutants indicated that the phosphorylation/dephosphorylation of 2C phosphatase proteins (PP2C) can...
be involved in ABA signaling during water stress (Yoshida et al. 2006). The mRNA level of several genes including APX, CAT, Superoxide dismutase (SOD), P5CS and gene for hydroxyproline biosynthesis for two sugarcane cultivars were compared and the results are presented in Table 1. The expression of Δ-pyrroline-5-carboxylate reductase, Δ-pyrroline-5-carboxylate synthase, ornithine–δ-ammonotransferase and γ-aminobutyric acid, all involved in the proline biosynthesis are up-regulated in tolerant cultivars while they remain down-regulated in the sensitive cultivars, those findings indicate that proline levels increase in the tolerant cultivars as an answer to water deficit even under mild stress condition, enhancing as the stress become severe. For the sensitive cultivar instead the same genes are down regulated showing that plants are not producing proline in order to react to water deficit. Genes involved in the antioxidative burst, antioxidant defenses, ascorbate peroxidase and catalase to overcome the oxidative burst with peroxidation of cell and sub cell membranes are not activated in the tolerant cultivars but are up regulated in the sensitive. Overall results suggest that a coordinated mechanism of plant protection starts with an increase in cytosolic Ca²⁺ level, increase in proline content that will prevent the formation of reactive oxygen species in tolerant cultivar with no need to activate the antioxidant defense system, while in the sensitive cultivar the osmoprotection system is not activated and cells will need to up regulate ROS-eliminating systems such as superoxide dismutase, ascorbate peroxidase and catalase to overcome the stress effects (Fig. 1).

CONCLUSION

Based on the observed results, when tolerant and sensitive cultivars were compared, it is possible to conclude that the tolerance to water deficit in sugarcane cultivars may be related to the early perception of stress signal mediated by the calcium intracellular level. This early perception will lead to the induction of calmodulin, kinases and phosphatases, which will in turn induce the up regulation of genes involved in the stress response. Considering that calcium (Ca²⁺) functions as a second messenger in signal transduction and the transient Ca²⁺ influx into the cell cytoplasm will stimulate stomatal closure and the ion transport pathway, this signal transduction will increase ABA content and consequently the induction of transcriptional factors like ABRE, stomatal closure, polyamine synthesis (increasing proline synthesis) and the entire cellular response to drought. Contrastingly for the sensitive cultivar, the results indicated that they exhibit a late perception of stress. There is no transient increase of calcium influx into the cell cytoplasm; no osmoprotection induced by increased levels of proline or GABA and the cells become exposed to an oxidative burst with peroxidation of cell and sub cell membranes and also the activation of the antioxidant defense system.

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