Plant Stress ©2013 Global Science Books

Osmoprotection in Sugarcane under Water Deficit Conditions

Gisele Cristina Dedemo[2] • Fabiana Aparecida Rodrigues[2] • Patricia Garnica Roberto[1] • Cyro Bueno Neto[1] • Suzelei de Castro França[1*] • Sonia M. Zingaretti[1]

[1] Unidade de Biotecnologia, Universidade de Ribeirão Preto, São Paulo, Brazil
[2] Departamento de Tecnologia, Universidade Estadual Paulista, São Paulo, Brazil
Corresponding author: * szingaretti@unaerp.br

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 mechanism 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

CONTENTS

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

Received: 31 January, 2012. Accepted: 25 December, 2012.

Invited Mini-Review

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 content builds up in many plant species under a wide range of biotic and abiotic stresses (Hare and Cress 1997; Xiong and Zhu 2002; Ábrahám *et al*. 2003; Deuschle *et al*. 2004; Claussen 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 is the only molecule 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 *et al.* 2002; Claussen 2005; Kishor *et al*. 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 synthesis 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 tolerant cultivars. It is known that a signal transduction pathway starts with signal perception, followed by the generation of secondary messengers like Ca^{2+} (Bethke *et al*. 1995). The modulation of Ca^{2+} levels, frequently initiate a protein phosphorylation cascade, which finally reaches target-proteins directly involved in cell protection of transcription factors controlling specific groups of stress-regulated genes (Xiong *et al*. 2002). One of the first responses to stress due to low temperature, drought and salinity in plant cells, is the transient increase in cytosolic calcium, derived from the influx of apoplastic reserve or the release from internal compartments such as the vacuole and endoplasmic reticulum, which contain higher levels of calcium than the cell cytosol (Buchanan *et al*. 2000; Xiong *et al*. 2002).

However, information regarding the nature of the signal transduction pathway that links perception of osmotic stress to building up of proline is very limited. The process of signals induced by stress could involve a reduction in turgor pressure, changes to phytohormone levels, and in free cytosolic calcium ($[Ca^{2+}]c$). An association between abscisic acid (ABA) and proline build up seems to be non-existent in tomatoes, though it has been suggested for some investigated plant species (Claussen 2005). Shah *et al*. (2001) studied the effects of calcium on proline build up 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.

Ca^{2+} 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 Ca^{2+} 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 a Ca^{2+} channel blocker was used. In maize suspension cultured cells the cytosolic calcium content increased from 25 to 150 μM, under O_2 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 protein turnover. In plants, the expression of genes that code for key-enzymes of proline biosynthesis, through amino acid glutamate as precursor P5CS (EC2.7.2.11/1.2.1.41) Δ-pyrroline-5-carboxylate-synthetase and P5CR (EC1.5.1.2) 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 (Claussen 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 1-semialdehyde (GSA) by GSA dehydrogenase NADPH-dependent (GSA redutase). This intermediary is spontaneously converted to pyrroline-5-carboxylate, which is reduced to proline by Δ-pyrroline-5-carboxylate reductase (P5CR), also NADPH-dependent. All these reactions for the synthesis of proline, with glutamate as precursor, occur in the cytoplasm. However, proline synthesis from ornithine is mediated by ornithine--δ-aminotransferase enzyme (OAT, EC2.6.1.13), which produces proline through the synthesis of Δ-pyrroline-5-carboxylate as an intermediary (Buchanan *et al*. 2000). Transamination of ornithine into P5C occurs in the mitochondria, unlike proline synthesis from glutamate, which takes place in the cytosol. Molecular studies suggest that the choice of pathway for the biosynthesis of proline is dependent on plant nitrogen levels (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 P5C 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 the same conditions that induce proline biosynthesis in the cytoplasm (Taylor 1996).

Prolyl hydroxylase (PH, EC1.14.11.2) 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 as modulator to increase its activity (Davey *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 γ-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 intensely 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 Krebbs cycle, nitrogen metabolism, and protection against oxidative stress, osmoregulation, and cell signaling (Bouché and Fromm 2004).

It has been suggested that GABA synthesis induced by stress is the 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, photorespiration 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 environment injuries to which plants are exposed, such as temperature, oxygen deprivation (Blokhina *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, alternating 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; Blokhina *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 the 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; Blokhina *et al.* 2003; Sairam 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 including ascorbate (ASC), glutathione, 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+ → 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 H_2O_2 is generated under normal metabolism; by electron transport in mitochondria and photorespiration in peroxisomes (Neill *et al.* 2002). This hemeprotein catalyzes the reaction 2 H_2O_2 → O_2 + 2 H_2O (Gupta *et al.* 1993; Netto 2001). Plant catalases derive from a common ancestor gene and can be divided into three distinct groups (CAT1, CAT2, and CAT3). Moreover, similar clusters of catalases 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 (H_2O_2) into water and molecular oxygen. These enzymes have extremely high catalytic levels, but low affinity with the substrate, since the reaction requires two H_2O_2 molecules to activate its site. An alternative way of breaking down H_2O_2 is through peroxidases, which are found in every cell and have high affinity with H_2O_2 compared to CAT. Peroxidases, however, require a reducer since they reduce H_2O_2 to water. In plant cells, the most important reduced substrate for H_2O_2 detoxification is ascorbate. APX uses two ascorbate molecules to reduce H_2O_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 monodehydroascorbate 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 transcriptomes, 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.

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

Description	Fold change			
	SP83-2847		SP90-1638	
	Mild	Severe	Mild	Severe
Proline biosynthesis				
putative P5CS	1.60	2.48	-2.05	-2.67
P5CS		2.44	-2.63	-2.39
P5CR	1.61	2.69	-1.81	-2.59
putative δ-OAT		-1.91		2.03
putative δ-OAT			-1.44	
Antioxidant defense systems				
Mn-SOD			0.67	
SOD[Cu-Zn] 2		-1.41	0.98	1.30
chloroplastic iron-SOD		2.58		-3.62
peroxisome type APX	-3.05	-2.82	2.35	2.69
APX	-0.95		0.60	
thylakoid-bound APX			1.55	
stromal APX			1.35	
cytosolic APX	-1.88		1.71	1.49
catalase-1		-2.20		1.86
MDHAR				
DHAR				
GABA biosynthesis				
putative GAD isozyme	1.44	1.40	-1.19	-1.42

Mild /severe are levels of stress
Original data

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. (Rodríguez-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

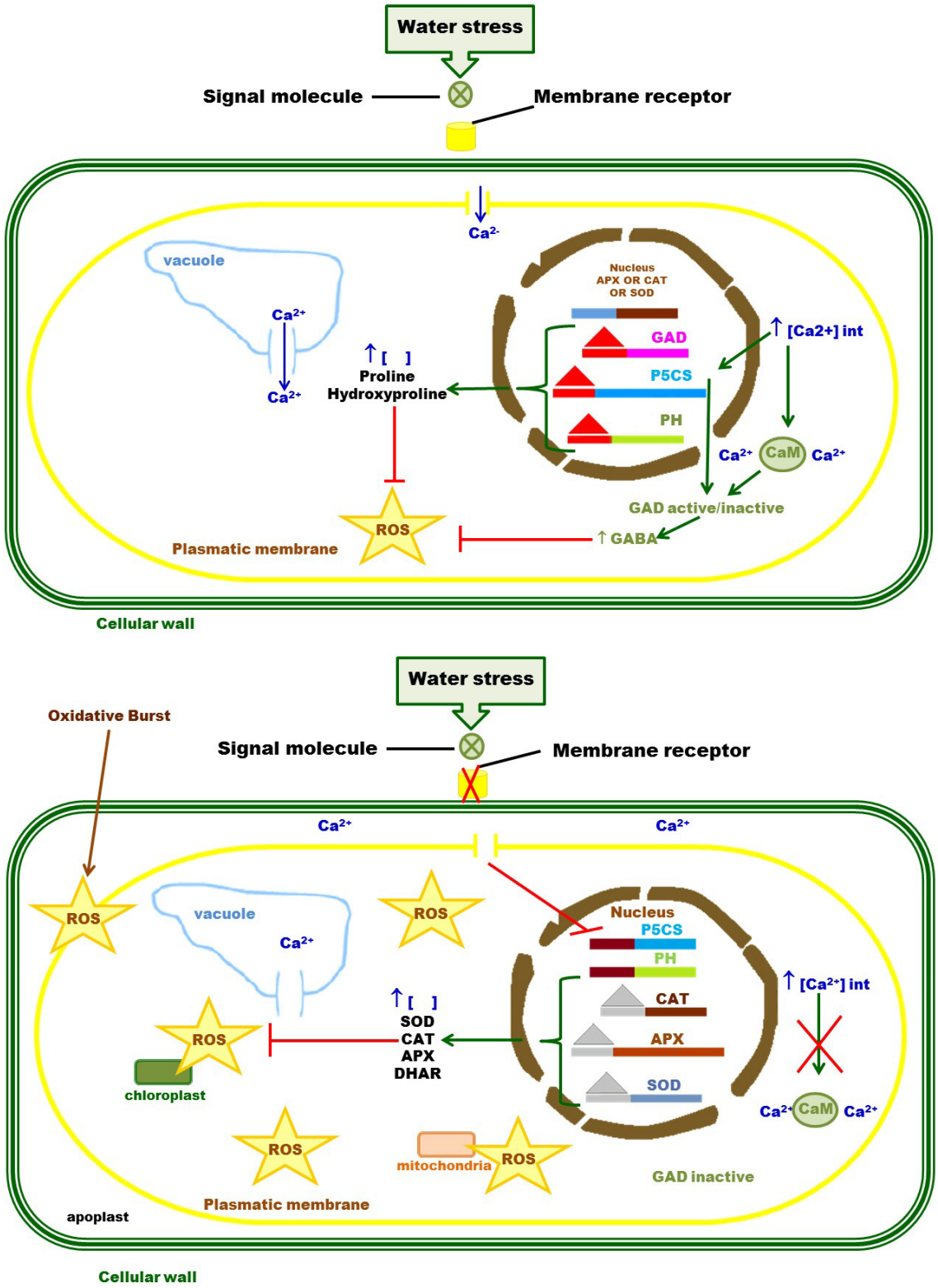

Fig. 1 Coordinated response mechanisms of tolerant (top) and sensitive (bottom) sugarcane cultivars to water stress. Calcium acting as a second messenger in water stress response. The transient Ca^{2+} influx into the cell cytoplasm will stimulate cell answers and this signal transduction will increase proline synthesis ABA content and the induction of transcriptional factors like ABRE and the entire cellular answer to drought in tolerant cultivars. Sensitive cultivars seem to have no perception of the stress signal which activates the transient Ca^{2+} influx into the cell cytoplasm and the correlated activation of the osmoprotection system inducing ROS production and the consequent expression of genes related to antioxidant enzymes.

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-aminotransferase 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 antioxidant systems 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 Ca^{+2} 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 (Ca^{2+}) functions as a second messenger in signal transduction and the transient Ca^{2+} 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.

ACKNOWLEDGEMENTS

This work was sponsored by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

REFERENCES

Ábrahám E, Rigó G, Székely G, Nagy, R, Koncz, C, Szabados L (2003) Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in *Arabidopsis*. *Plant Molecular Biology* **51**, 363-372

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* **55**, 373-399

Arora A, Sairam RK, Srivastava GC (2002) Oxidative stress and antioxidative system in plants. *Current Science* **82 (10)**, 1227-1238

Bethke PC, Gilroy S, Jones RL (1995) Calcium and plant hormone action. In: Davies PJ (Ed) *Plant Hormones: Physiology, Biochemistry and Molecular Biology* (2nd Edn), Kluwer Academic Publishers, Dordrecht, pp 298-317

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany* **91**, 179-194

Bouché N, Fromm H (2004) GABA in plant: Just a metabolite? *Trends in Plant Science* **9 (3)**, 110-115

Bray EA (2002) Classification of genes differentially expressed during water-deficit stress in *Arabidopsis thaliana*: An analysis using microarray and differential expression data. *Annals of Botany* **89**, 803-811

Buchanan BB, Gruissem W, Jones RL (2000) *Biochemistry and Molecular Biology of Plants*, American Society of Plant Physiologists, Rockville, Maryland, pp 1158-1201

Chaudhary B, Hovav R, Flagel L, Mittler R, Wendel JF (2009) Parallel expression evolution of oxidative stress-related genes in fiber from wild and domesticated diploid and polyploidy cotton (*Gossypium*). *BMC Genomics* **10**, 378

Chen S, Polle A (2010) Salinity tolerance of *Populus*. *Plant Biology (Stuttgart)* **12 (2)**, 317-333

Chen THH, Murata N (2002) Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Current Opinion in Plant Biology* **5**, 250-257

Chinnusamy V, Schumaker K, Zhu J (2004) Molecular genetic perspectives on cross-talk and specificity in abiotic stress signaling in plants. *Journal of Experimental Botany* **55 (395)**, 225-236

Claussen W (2005) Proline as a measure of stress in tomato plants. *Plant Science* **168**, 241-248

Davey MW, Van Montagu M, Inzé D, Sanmartin M, Kanellis A, Smirnoff N, Benzie IJJ, Strain JJ, Favell D (2000) Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture* **80**, 825-860

Deuschle K, Funck D, Forlani G, Stransky H, Biehl A, Leister D, van der Graaf E, Kunze R, Frommer WB (2004) The role of Δ1-pyrroline-5-carboxylate dehydrogenase in proline degradation. *Plant Cell* **16**, 3413-3425

Garcia GO, Ferreira PA, Miranda GV, Neves JCL, Moraes WB, Santos DB (2007) Leaf contents of cationic macronutrients and their relationships with sodium in maize plants under saline stress. *Idesia* **25 (3)**, 93-106

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* **48 (12)**, 909-930

Guan LM, Zhao J, Scandalios JG (2000) *Cis*-elements and trans-factors that regulate expression of the maize Cat1 antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signaling molecule for the response. *Plant Journal* **22 (2)**, 87-95

Guimarães RS, Mutton MA, Mutton MJR, Ferro MIT, Ravanel GC, Silva JA (2008) free proline accumulation in sugarcane under water restriction and spittlebug infestation *Science Agricola* **65 (6)**, 628-633

Gupta AS, Webb RP, Holaday AS, Allen RD (1993) Overexpression of superoxide dismutase protects plants from oxidative stress. *Plant Physiology* **103**, 1067-1073

Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regulation* **21**, 79-102

Hernández JA, Jiménez A, Mullineaux P, Sevilla F (2000) Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defenses. *Plant, Cell and Environment* **23**, 853-862

Hoekstra FA (2002) Essay 11.5: Role of respiration in desiccation tolerance. In: *Plant Physiology* (3thEd), Sinauer Associates Inc., Sunderland, MA, 690 pp

Iyer S, Caplan A (1998) Products of proline can induce osmotically regulated genes in rice. *Plant Physiol*ogy **116**, 203-211

Jain M, Nandwal AS, Kundu BS, Kumar B, Mann A, Kukreja S (2006) Water relations, activities of antioxidants, ethylene evolution and membrane integrity of pigeonpea roots as affected by soil moisture. *Biologia Plantarum* **50 (2)**, 303-306

Jiang M, Zhang J (2002) Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *Journal of Experimental Botany* **53 (379)**, 2401-2410

Kido EA, Neto JRCF, Silva RLO, Pandolfi V, Guimarães ACR, Veiga DT, Chabregas SM, Crovella S, Benko-Iseppon AM (2012) New insights in the sugarcane transcriptome responding to drought stress as revealed by supersage. *The Scientific World Journal* **2012**, 1-14

Kishor PB, Sangam S, Amrutha RN, Sri Laxmi P, Naiduu KR, Rao KRSS, Sreenath Rao, Reddy KJ, Theriappan P, Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Current Science* **88 (3)**, 424-438

Kwon SY, Jeong YJ, Lee HS, Kim JS, Cho KY, Allen RD, Kwak SS (2002) Enhanced tolerances of transgenic tobacco plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against methyl viologen-mediated oxidative stress. *Plant, Cell and Environment* **25**, 873-882

Maggio A, Miyazaki S, Veronese P, Fujita T, Ibeas JI, Damsa B, Narasimhan ML, Hasegawa PM, Joly RJ, Bressan RA (2002) Does proline accumulation play an active role in stress-induced growth reduction? *Plant Journal* **31 (6)**, 699-712

Nayyar H (2003) Accumulation of osmolytes and osmotic adjustment in water-stressed wheat (*Triticum aestivum*) and maize (*Zea mays*) as affected by calcium and its antagonists. *Environmental and Experimental Botany* **50**, 253-

264

Neill S, Desikan R, Hancock J (2002) Hydrogen peroxide signaling. *Current Opinion in Plant Biology* **5 (5)**, 388-395

Nepomuceno AL, Neumaier N, Farias JRB, Oya T (2001) Tolerância à seca em plantas. *Biotecnologia, Ciência e Desenvolvimento* **23**, 12-18

Netto LES (2001) Oxidative stress response in sugarcane. *Genetics and Molecular Biology* **24 (1-4)**, 93-102

Noctor G, Foyer CH (1998) Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**, 249-279

Oda A, Shimizua M, Kuroha T, Satoh S (2005) Induction of xylem sap methylglycine by a drought and rewatering treatment and its inhibitory effects on the growth and development of plant organs. *Physiologia Plantarum* **124**, 515-523

Ozturk ZN, Talamé V, Deyholos M, Michalowski CB, Galbraith DW, Gozukirmizi N, Tuberosa R, Bohnert HJ (2002) Monitoring large-scale in transcript abundance in drought- and salt-stressed barley. *Plant Molecular Biology* **48**, 551-573

Papini-Terzi FS, Rocha FR, Vêncio RZ, Nicoliello OKC, Felix JDM, Vicentini R, Rocha CS, Simões ACQ, Ulian EC, Di Mauro SMZ, Silva AM, Pereira CAB, Menossi M, Souza GM (2005) Transcription profiling of signal transduction-related genes in sugarcane tissues. *DNA Research* **12 (1)**, 27-38

Rabbani MA, Maruyama K, Abe H, Khan AM, Katsura KI, Yoshimara K, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. *Plant Physiology* **133**, 1755-1767

Raymond MJ, Smirnoff N (2002) Proline metabolism and transport in maize seedlings at low water potential. *Annals of Botany* **89**, 813-823

Rocha FR, Papini-Terzi FS, Nishiyama MY, Vêncio RZN, Vicentini R, Duarte RDC, Rosa JrVE, Vinagre F, Barsalobres C, Medeiros AH, Rodrigues FA, Ulian EC, Zingaretti SM, Galbiatti JA, Almeida RS, Figueira AVO, Hemerly AS, Silva-Filho MC, Menossi M, Souza GM (2007) Signal transduction-related responses to phytohormones and environmental challenges in sugarcane. *BMC Genomics* **8 (71)**, 1-22

Rodrigues FA, Graça JP, Laia ML, Nhani Jr. A, Galbiati JA, Ferro MIT, Ferro JA, Zingaretti SM (2011) Sugarcane genes differentially expressed during water deficit. *Biologia Plantarum* **55 (1)**, 43-53

Rodrigues FA, Laia ML, Zingaretti SM (2009) Analysis of gene expression profiles under water stress in tolerant and sensitive sugarcane plant. *Plant Science* **176 (2)**, 286-302

Rodríguez-Serrano M, Bárány Y, Prem D, Coronado MJ, Risuenõ MC, Testillano OS (2012) NO, ROS and cell death associated with caspase-like activity increase in stress-induced microspore embryogenesis of barley. *Journal of Experimental Botany* **63 (5)**, 2007-2024

Sairam RK, Tyagi A (2004) Physiology and molecular biology of salinity stress tolerance in plants. *Current Science* **86 (3)**, 407-421

Serrano R, Montesinos C (2003) Molecular bases of desiccation tolerance in plant cells and potential applications in food dehydration. *Food Science and Technology International* **9 (3)**, 157-161

Shah SH, Tobita S, Shono M (2001) Supplemental calcium regulates proline accumulation in NaCl-stressed suspension cultures of *Orysa sativa* L. at the level of mRNA translation. *Pakistan Journal of Biological Sciences* **4 (6)**, 707-710

Shelp BJ, Bown AW, McLean MD (1999) Metabolism and functions of gamma-aminobutyric acid. *Trends in Plant Science Reviews* **4 (11)**, 446-452

Subaiah CC, Bush DS, Sachs MM (1994) Elevation of cytosolic calcium precedes anoxic gene expression in maize suspension cultured cells. *The Plant Cell* **6**, 1747-1762

Taylor CB (1996) Proline and water deficit: ups, downs, ins, and outs. *The Plant Cell* **8**, 1221-1224

Xiong L, Schumaker KS, Zhu J-K (2002) Cell signaling during cold, drought, and salt stress. *Plant Cell* **8**, 165-183

Yoshida R, Umezawa T, Mizoguchi T, Takahashi S, Takahashi F, Shinozaki K (2006) The regulatory domain of SRK2E/OST1/SnRK2.6 interacts with ABI1 and integrates abscisic acid (ABA) and osmotic stress signals controlling stomatal closure in Arabidopsis. *The Journal of Biological Chemistry* **281 (8)**, 5310-5318

Zingaretti SM, Rodrigues FA, Graça JP, Pereira LM, Lourenço MV (2011) Sugarcane responses at water deficit conditions. In: Ismail Md, Rahman M, Hasegawa H (Eds) *Plant Stress*, Intech, Croatia, pp 255-276