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1. Introduction

Sugarcane (*Saccharum* spp.), a plant of the Poaceae family, is an important source of sucrose and ethanol in many tropical and sub-tropical countries. It was introduced in Brazil during the colonization period, and today represents one of the main cultures of the economy. Brazil is currently the world’s largest sugarcane producer. The 08/09 crop yielded approximately 569 millions of tons, totaling 27.5 million liters of ethanol and 31.0 million tons of sugar (ÚNICA, 2011). This makes Brazil responsible for providing 25% of the sugar consumed all over the globe (ÚNICA, 2011), sharing with the United States the leadership in ethanol production. The search for alternative, renewable sources of energy, placed the spotlight on Brazilian ethanol, causing an increase in the production of bi-fuel automobiles, which estimates foresee will represent 85% of the market in a few years.

Today, in Brazil, more than 9 million ha are currently used to cultivate sugarcane, but the demand for sugar and ethanol will have it increased to 64 million ha by the years 2018/2019. The growing demand for ethanol leads to an increase in production, which subsequently demands expansion of cultivated areas as well as the search for new technologies which will enable higher agricultural and industrial productivity. The current occupied agricultural area is reportedly, only 2.4%, and expansion has occurred in regions of the country where this culture will need adjustments due to soil and weather characteristics.

Plants are exposed to adverse environmental conditions, and drought is the major abiotic factor that can damage its growth and development. Drought also limits the areas suitable to agriculture. It is known that, as for any crop, during vegetative growth water is essentially required to obtain maximum yield and drought events in this stage can significantly decrease productivity.

Sugarcane is among the crops which produce a higher amount of biomass per unit of cultivated area and water requirement varies throughout the developing stages, thus for higher tillering and development of culms, there is a higher water requirement than during the maturation stage, when this need is diminished.

In some countries as Australia, Sudan and South Africa about 60% of sugar produced is grown in irrigated areas (Inman-Bamber & Smith, 2005), a practice which always results in production cost increase.
Plants under water deficit suffer a disturbance on cellular homeostasis and must change their metabolism in order to protect themselves against stress. Some plants can undergo an acclimation process and delay effects of stress by using the water available in the soil to slow the process. The level of drought tolerance exhibited by plants can be evaluated by growth analysis and plant productivity under water stress conditions. Drought tolerance is a complex process that varies according to the severity of stress, age of the plant and the water use efficiency. Moreover, other abiotic or biotic stresses that might occur simultaneously can become more stressful to plants. Some cultivars can tolerate the stress more effectively than others. As well as the empirical observations performed in field during growing seasons, the first investigation includes the evaluation of physiological parameters that can indicate more tolerant or sensitive plants. Once the tolerance level is known, cultivars with different drought tolerance levels can be used in gene expression studies to investigate the molecular basis of tolerance.

2. Water deficit

The growing food production demand has led to an agricultural area increase involving areas never before cultivated and frequently poor in nutrients and water deficient. Moreover, climate changes have been directly affecting water availability globally, due to variations in precipitation levels which estimate serious consequences for the water balance in plants (Ryan, 2011). Correspondingly, drought is among the greatest limits to productivity and geographic distribution of the cultures (Fisher & Turner, 1978; Le Rudulier et al., 1984; Delauney & Verma, 1990).

It is well known that water is a very important solvent, essential for all types of living cells because it is directly related to various growth, development and reproductive metabolic functions. Plants are constituted of up to 85% - 95% of water and any losses in water content produce changes in physiological and biochemical cellular reactions (Sawhney & Sing, 2002). Water deficit may be considered as a deviation in the optimal condition to life, causing changes and responses in all functional levels of the organism, which are primarily reversible, but may become permanent and is frequently observed in many crops, with effects varying according to species, duration of stress, season and severity. Water deficit can trigger a negative impact upon growth and development of the crop, compromising plant productivity especially if the drought duration exceeds the capacity of drought-tolerance of the plant species (Inman-Bamber, 2004; Smit & Singles, 2006). Regardless of the temporary nature of the stress, the set of properties and vital functions of the plant gradually decrease according to its duration (Larcher, 2004).

While moderate water deficit causes significant morphological and physiological changes in plants (Creelman et al., 1990), severe deficit may lead to plant death (Cheng et al., 1993). Among the physiological effects are changes in a variety of processes such as radiation capture, foliar temperature, stomatal conductance, transpiration, photosynthesis and respiration, which determine culture productivity (Qing et al., 2001, Silva et al., 2007). On a cellular level, water deficit occurs when transpiration rates exceed water absorption, being the result of different types of stress and potentially leading to changes in concentration of solutes, cell volume and shape of membrane, changes in water potential gradients, loss of cell turgor, compromising of membrane integrity, protein denaturation, total loss of free water leading to dryness and dehydration (Bray, 1997).

Hence, a decrease in water potential is directly related to the reduction in plant photosynthesis rates, where during water stress less carbon assimilation occurs, resulting in
insufficient dissipation of electrons generated by the electron transfer chain and subsequently an overproduction of reaction oxidative species (ROS) (Edreva, 2005). Other abiotic stresses may be related to water stress acting as limiting factors in production, such as salinity and temperature extremes (Bray, 2004). Therefore, these stressing factors prevent plants from performing to their full genetic potential (Zhu, 2002). Albert et al., (2011), studied the interaction of adverse environmental effects, namely CO₂ concentration increase, high nocturnal temperature and water stress, as to photosynthesis capacity. They observed that estimated future weather conditions will essentially affect physiological processes of plants in unforeseeable ways regarding isolated factors; though dependant on the interaction of growth strategy of the species and their capacity to adjust to the conditions of water availability in the soil.

Other studies indicate that the effects of drought, salinity, temperature extremes and oxidative stress are interconnected and may induce similar damage on a cellular level, such as osmotic changes resulting in homeostasis interruption and ion distribution in the cell (Zhu, 2001; Wang et al., 2007). Accordingly, it is clear that abiotic stresses occur normally as a pool of factors which interact and that plants react to these environmental adversities in a complex manner (Shao et al., 2007).

The interaction of water with other environmental factors can be explained by the interconnectivity of plant, soil and the atmosphere. Transpiration occurs as a response to certain atmospheric conditions that determine a gradient of water under continuous tension, or total potential difference, from its highest point to the surface of the root interacting with the soil, which promotes ascension and soil water absorption. However, low soil humidity affects absorption, so when atmospheric demand increases and the soil is not able to produce enough water, the plant loses more water, there is a delay in absorption and water stress is established (Pires et al., 2010).

Generally, quick changes in water availability occur in superficial layers of the soil, and many studies report that seedling mortality is higher when compared to plant mortality under similar conditions (Schlesinger et al., 1982; Wellington, 1984), showing water absorption by plants occurs in extracts on soil profile according to the distribution of the radicular system and its water content (Pires et al., 2010). Therefore, we may conclude that adult plants with a well developed radicular system and deeply rooted are more drought tolerant. In this context, it was observed that plants can present mechanisms or strategies of tolerance and susceptibility that are expressed during their developmental stages (Osmond et al, 1097; Lichtenthaler, 1996; Chaves et al., 2003).

Different mechanisms and processes are involved in order to avoid the effects of drought and promote tolerance, among which phenology is considered one of the essential factors because water stress is highly variable in duration and severity (Witcombe et al., 2008). In regard to plant evolution, water availability is classified as the most relevant factor among elements which collaborate to abiotic stresses. Since signal transduction induces plant responses, one can infer that signals induced by stress are highly responsible for plant adjustment (Khu, 2002). Because water stress can lead to serious damage in plants, they developed complex transduction pathways to overcome injuries caused by stress (Fujita et al., 2005).

Plant changes aiming tolerance are normally based on manipulation of genes related to protection and maintenance of the structure and function of cells. In contrast to monogenetic characters of resistance to pests and herbicides, abiotic stresses involve more
complex genetic responses, and therefore are more difficult to control and manipulate. Genetic engineering involves the transfer of one or more genes involved in signal transduction, regulation, or those that codify functional and/or structural genes. Even though efforts to improve plant tolerance to stress through genetic engineering have resulted in significant findings, the nature of the genetic mechanisms in answer to stress makes these improvements extremely hard (Wang et al., 2003). Many studies have been conducted for the understanding of the mechanisms of plant response to stress, in order to identify gene products that play a role on water stress tolerance (Bray, 2004). Thus, elucidation of the effects of water stress on plants in a broader, interdisciplinary context is increasingly relevant.

3. Physiological parameters in sugarcane

According to Taiz & Zeiger, (2006) current crop productivity in many regions is only partly due to the genetic potential of the plants. Water stress is the major factor that can limit the potential yield of crop plants by up to 70% (Gosal et al., 2009; Morison et al., 2008), and sugarcane is especially affected by drought. Sugarcane presents four developmental stages (Gascho & Shih, 1983), described as: 1) Germination: the development of buds and roots, taking from 30 to 35 days; 2) Tillering: issuance of secondary and tertiary tillers, beginning approximately on the fortieth day after planting and lasting up to 120 days; 3) Grand growth: tillers growth and development with height gain and basal sugar accumulation taking up to 9 months after planting; 4) Maturity: accumulation of photoassimilates and fast sugar synthesis, lasting until the harvesting period. Tillering and grand growth phases are known as critical stages of drought-sensitivity due to the high need of water for sugarcane growth (Ramesh, 2000). During these phases the relationship between water content and the respective physiological responses can be used to identify and distinguish sugarcane genotypes tolerant to drought (Endres et al., 2010).

To uptake CO₂, plants need to maintain stomata open (Taiz & Zeiger, 2006), but this process requires a high transpiration rate, which can be a limiting factor in some areas due to the low water availability (Molina, 2002). However, plants present morphological, biochemical and physiological mechanisms that allow the use of available water more efficiently (Azevedo et al., 2011; Ghannoum, 2009). The C4 metabolism of plants such as sugarcane can probably facilitate their development in hot and dry areas by reducing the photorespiration rate and water loss.

In plants with C4 metabolism (C4 plants) the first stable molecule produced from fixation of carbon dioxide (CO₂) is the 4-carbon dicarboxylic acid oxaloacetate (OAA), which is converted into malate or aspartate. Besides the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) present in bundle sheath cells, plants C4 also have the phosphoenolpyruvate carboxylase (PEPc, EC 4.1.1.31) in mesophyll cells. PEPc enzyme can increase CO₂ fixation due to its high affinity for CO₂ molecules. The spatial compartmentalization of these enzymes causes the fixed CO₂ to be translocated as malate and aspartate to the bundle sheath cells, where decarboxylation occurs with the input of carbon into the Clavin-Benson cycle (Lopes et al., 2011; Taiz & Zeiger, 2006).

In addition to the efficiency of CO₂ fixation, C4 plants may have three forms of decarboxylation, and sugarcane shows evidences suggesting the presence of two of them, NADP-malic (NADP-ME, EC 1.1.1.40) and the phosphoenolpyruvate carboxykinase (PCK, EC 4.1.1.49), in a process where one enzyme activity probably supplements the other.
(Christin et al., 2007; Ghannoum, 2009). Despite mechanisms that facilitate reactions of CO\textsubscript{2} fixation, sugarcane as any given plant species is subject to water deficit. Longer periods of drought on sugarcane crops can significantly decrease growth, productivity and quality of product (Wiedenfeld, 2000). In C4 plants some evidences demonstrate that photosynthesis is highly sensitive to water deficit (Ghannoum, 2009). Moreover, these plants present low recovery capacity mainly when water deficit exceeds the plant recovery capacity limiting the photosynthesis metabolic pathways (Ripley et al., 2010). In Brazilian sugarcane cultivars the capacity to recover the physiological responses was compromised from the tenth day of stress and damaged the photosynthetic apparatus, as can be observed through the low photosynthetic rate, stomatal conductance and transpiration rate (Graça et al., 2010). Plants under water deficit conditions show modifications in their metabolism to tolerate water loss. Concerning these changes, the root system is the first part to detect the stress and signal to other tissues. The hydraulic perturbation (Buckley, 2005) stimulates plants to send chemical signals through roots to trigger changes on stomata during the water deficit. Therefore, the abscisic acid (ABA) (Kholová et al., 2010), the pH (Schachtman & Goodger, 2008) and the ionic distribution (Bahrun et al., 2002) seem to play an important role on signaling throughout the plant under water stress. Molecular studies have identified a wide range of genes expressed by sugarcane plants under water stress conditions (Iskandar et al., 2011; Prabu et al., 2010; Rodrigues et al., 2011). Regarding the responses to stress, signaling pathways regulated by hormones are highly drought-responsive, mainly those associated with the increased ABA synthesis (Pinheiro & Chaves, 2011). Under drought stress condition, the level of endogenous ABA was increased and its function upon the stomatal closure can protect plants against immediate desiccation (Yoshida et al., 2006). In sugarcane cultivars some genes showed similarity to ABA-regulated proteins and genes directly or indirectly involved in its biosynthesis in plants submitted to water deficit, as well as a reduction in stomatal conductance (Rodrigues et al., 2011). Soil water content seems to be more influential in stomatal conductance than plant water content (Davies et al., 2002; Taiz & Zeiger, 2006). Sugarcane plants also presented a decreased soil water content under moderate (42%) and severe stress (22%) which produced changes in all photosynthetic apparatus, such as stomatal closure, reduction of transpiration and photosynthetic rate, as well as in RWC, photochemical efficiency of photosystem II (PS II), and increase in leaf temperature in plants submitted to water deficit (Rodrigues et al., 2009, 2011).

Different methods have been applied in plant genetic breeding programs, and considering the drought tolerance, programs have focused mainly on the characterization of genotypes under water stress conditions (Condon et al., 2004). Thus, the analyses of physiological parameters have allowed the selection and classification of cultivars through comparative tests using genotypes with known potential to drought tolerance or to drought sensitivity. Among the physiological parameters, the relative water content (RWC) is considered a fast and cheap tool to perform this type of physiological research in breeding programs (Matin et al., 1989; Silva et al., 2007). RWC represents an indicator of plant water balance because it expresses the absolute water amount the plant requires to reach artificial full saturation (González & González-Vilar, 2001). In fact, RWC indicates the level of cellular and tissues hydration which is important for the physiological plant metabolism (Silva et al, 2007). The control of physiological functions is related to plant water content and changes in RWC seem to directly affect all photosynthetic apparatus in sugarcane plants (Graça et al., 2010). When plants under water deficit start to lose water, RWC decreases and triggers a
significant reduction in the CO$_2$ uptake rate due to the stomatal closure (Buckley, 2005). In sugarcane a decrease of 10 to 20% in RWC caused reduction in all photosynthetic apparatus of tolerant and sensitive sugarcane plants submitted to water deficit (Graça et al. 2010). RWC applied to distinguish sugarcane genotypes demonstrates that tolerant cultivars show a higher percentage than sensitive plants, and the probable hydration of the protoplast in this cultivars can ensure it productivity in areas with low water availability (Silva et al., 2007).

In sugarcane the increase in the metabolites level (free proline, soluble sugars, glycinebetaine, soluble phenolic compounds, carotenoids and anthocyanins) was essential to improvement of net assimilation and heat tolerance (Wahid, 2007). Osmoprotectants are molecules that play roles in cell osmotic adjustment and due to their importance in stress events they are applied in breeding programs (Cha-um et al., 2008). However, biochemical and physiological evaluations of transgenic sugarcane improved with $\Delta^{1}$-pyrroline-5-carboxylate synthetase demonstrate that the increased proline biosynthesis was more related to reactive oxygen species (ROS) scavengers than to osmoprotector (Molinari et al., 2007). In sugarcane the accumulation of proline and the photosynthetic activity were used as effective indicators to select drought tolerant cultivars (Cha-um et al., 2008). Under salt stress and water deficit sugarcane plants seem to up the osmoprotectant proline synthesis in response to both stresses. In the same study, the photochemical efficiency of photosystem II, stomatal conductance and transpiration rate were also reduced as consequence of the stress (Cha-um et al., 2008). Photosynthetic rate measured in plants under drought stress can present variation according to species and severity of stress. *Eragrostis curvula* submitted to water stress showed higher photosynthetic rate and RWC than the ones observed in tolerant cultivar (Colom & Vazzana, 2003). In sugarcane under dehydration conditions low photosynthetic rates at moderate (8 days) and severe stress (10 days) were observed (Rodrigues et al., 2009, 2011). Submitting the same sugarcane cultivars to other water-limited regime where irrigation was completely suppressed, produced low photosynthetic rates under a moderate stress. Both sensitive and tolerant plants exhibited decreased photosynthesis, although under normal water irrigation tolerant sugarcane plants showed higher photosynthetic activity compared to sensitive plants (Graça et al., 2010).

In addition to damages caused by water deficit, the stressed plant can also suffer the effects of a secondary stress, such as oxidative stress, as a consequence of the first stressful situation. Accumulation of reactive oxygen species [singlet dioxygen (O$_2^•$) and superoxide (O$_2•^-$)] occurs naturally during the electron transport in photosynthesis reactions (Miller et al., 2010). As plants close the stomata under water deficit and reduce the internal CO$_2$ concentration, the generation of reactive oxygen species seems to stimulate mechanisms that reduce oxidative stress and so it may play an important role in drought tolerance (Arora et al., 2002).

In tylakoid membranes, photosystems I and II capture photons from sunlight and convert light energy into chemical energy by using water as base in this biochemical process. Reduction in water availability can produce low efficiency on photosystem II, consequently few molecules of ATP and NADPH are produced, reducing the CO$_2$ fixation (Souza et al., 2004; Taiz & Zeiger, 2006). Under normal water resources, sensitive and tolerant genotypes presented few differences in photochemical efficiency (PSII). Nonetheless, the efficiency on photochemical efficiency (PS II) determined by using drought-stressed sugarcane, showed variations between the sensitive and tolerant cultivars, where the tolerant plants exhibited better use of the photosynthetic apparatus. Based on this result, it is suggested that tolerant plants can maintain the oxidative process at normal levels in photochemical efficiency (PSII).
II), differently than the sensitive cultivar (Graça et al., 2010). In general water deficit decreases the photochemical efficiency (PS II), and the ability of the cultivar to maintain a high level of $F_v/F_m$ can be an indicative of the radiation use efficiency and carbon assimilation, which has become a promising tool to select cultivars more tolerant to drought (Silva et al., 2007).

Maintenance of leaf temperature requires large amounts of water transpired throughout the plant in order to keep it under the ambient temperature and the appropriate functioning of photosynthetic apparatus (Machado & Paulsen, 2001). Under high temperatures (above 32°C) sugarcane cultivars limit the internode growth and reduce the sucrose content (Bonnett et al., 2006). In addition, the acclimation process during the cooling also includes small size of plant, leaf orientation, leaf rolling that minimize the area exposed to the environment (Taiz & Zeiger, 2006). Leaf rolling in sugarcane plants is described as a sensitivity characteristic, however it can be understood as part of the acclimation process used by plants to limit the leaf surface area and then avoid water deficit rather than endure it (Inman-Bamber & Smith, 2005). Under high temperatures, photosynthesis and respiration are inhibited mainly by the reduction of cell membranes stability. Damages in photosynthesis are more closely related to changes in membrane properties and with the decoupling of the mechanisms of energy transfer in chloroplasts than to a protein denaturation (Prasad et al., 2008; Sage & Kubien, 2007). As a C4 plant, sugarcane exhibits its highest productivity under temperatures of 30 to 34°C. Since the plant hydric status is not compromised, high temperature seems not to be a limiting factor either to the photosynthetic capacity or to wheat and sorghum development (Machado & Paulsen, 2001). Nevertheless, in drought-stressed sugarcane cultivars the increase of leaf temperature occurred due to the reduction in transpiration, which was triggered by stomatal closure (Graça et al., 2010). In tolerant plants, a higher water status seems to support the stomatal aperture and to maintain the leaf cooling (Silva et al., 2007). Sugarcane cultivars respond differently to water deficit. The tolerant cultivar CTC15 showed a reduction of 4% in TRA, which probably produced the stomatal closure that consequently increased leaf temperature. In the tolerant cultivar SP83-2847, the increase in leaf temperature was significant only when RWC was reduced to 20% in stressed plants (Graça et al., 2010). In sugarcane, signaling between roots and leaves, that leads to stomatal closure, seems to be more closely related to water availability in soil than to leaf water potential (Inman-Bamber & Smith 2005; Smit & Singels, 2006).

Thus, RWC, photochemical efficiency (PS II), stomatal conductance and the photosynthetic rate are some physiological parameters that have been useful in characterizing genotypes tolerant to drought (Buckley 2005; Vinocur & Altman, 2005; Shao et al., 2008; Tezara et al., 2008). Physiological parameters and the identification of genes can be applied as a base for research and development of new sugarcane cultivars (Hotta et al., 2010). Some physiological and biochemical methods used to select cultivars sensitive and tolerant to water deficit in breeding programs have showed promising results. They have also shown a wide applicability based on the low cost of some tools such as RWC as well as the availability of data (Azevedo et al., 2011; Silva et al., 2007).

4. Genetic analysis

Long periods of drought can be tolerated by plants, but the ability to maintain growth and development under limited water resource is considered a characteristic of tolerance to
water deficit. Molecular biology, associated with classic genetic breeding programs, became an important tool to detect genetic variability by reducing the time and maximizing the efficiency. The identification and characterization of genes involved in drought tolerance brings knowledge about the perception of the stress and how plants respond to this adverse condition. Concerning this, studies have been performed aiming to identify new sources of variability in different crops (Cramer et al., 2007; Micheletto et al., 2007; Poroyko et al., 2007; Zhuang et al., 2007).

Drought is firstly detected by root tissues. Once roots detect a decrease in soil water content it emits a signal to leaves triggering the stomatal closure (Taiz & Zeiger, 2006). Synthesis of endogenous ABA might be related to signaling between plant tissues as other chemical signals (Schachtman & Goodger, 2008). Growth of the primary root is stimulated; probably regulated by the increase in abscisic acid (ABA) content. Plants subjected to water deficit lose the integrity of membranes and integral proteins (Larcher, 2003). As the severity of water stress increases, the photosynthetic rate decreases and the cell metabolism homeostasis becomes unbalanced. The plant hormone ABA appears to play an important role in protecting cell and signaling the expression of some stress-responsive genes (Shinozaki & Yamaguchi-Shinozaki, 2007).

The early perception of water stress as well as the signal transduction is very important to plant response to adverse environment conditions. Protein kinases, phosphatases and calmodulins are directly involved in a complex cell communication process (Yoshida et al., 2006). In plant responses to stress, morphological, physiological, biochemical and molecular changes are triggered to protect plants against desiccation (Rachmilevitch, 2006). In this context, many genes are induced in order to maintain the cell water content (osmoprotectants), to facilitate the water and solutes transport (water channel proteins) or to prevent the reactive oxygen species (ROS) (Gorantla et al., 2007). Drought stress-responsive genes can be divided into two categories: genes encoding functional proteins or genes encoding regulatory proteins. The functional group includes proteins such as water channel, transporters, detoxification enzymes, chaperones or proteases. The regulatory genes involve proteins whose roles are related to signaling and transcription factors (Shinozaki & Yamaguchi-Shinozaki, 2007).

In general, all biological processes are affected by water deprivation; however plants under water stress are more susceptible to other stresses (Zhuang et al., 2007). Temperature is one of the factors that can enhance the effect of water stress on plants. Under water stress plants close the stomata to avoid the water loss to atmosphere and can increase the cell temperature. Heat stress also inhibits the photosynthesis and decreases the stability of membranes as well as increasing the cell respiration. Plants subjected to heat stress express heat shock proteins, molecules involved in protecting enzymes and structural proteins against denaturation and protein aggregation (Rizhsky et al., 2004; Taiz & Zeiger 2006), they are often identified in drought-stressed tissues.

The Brazilian Sugarcane Expressed Sequence Tag (ESTs) Sequencing Project (Sucest project) was pioneer in sugarcane functional studies (Vettore et al., 2003). Around 238,000 ESTs were produced from plants of different vegetative or reproductive stages grown in vitro and in vivo under diverse conditions. Data obtained from this project was used to support studies of biotic (Barsalobres-Cavallari et al., 2006) and abiotic stresses (Kurama et al., 2002; Nogueira et al., 2003), as well as other researches with sugarcane (Camargo et al., 2007; Papini-Terzi et al., 2005; Rocha et al., 2007; Rosa et al., 2005). To perform a collection of ESTs from Brazilian sugarcane cultivars expressed specifically under water deficit conditions, the data provided by...
Sucrose project was also applied in a large scale gene expression study using sugarcane cultivars with different tolerance to drought (Rocha et al., 2007; Rodrigues et al., 2009, 2011). After the advent of sequencing projects and the consequent establishment of databases, the use of some molecular techniques became a valuable tool in identifying genes involved in plant responses to stress. To measure gene expression under drought conditions, 3,575 cDNA clones from leaf libraries generated by the Sucrose project were used through the DNA macroarray technique. This method was chosen due to its sensibility to detect expression at low levels. In addition, macroarray has been a useful tool for transcriptional studies such as those to investigate the behavior of plants under salinity (Merchan et al., 2007) or water stress (Becker et al., 2006; Maraschin et al., 2006), to study plant hormone regulation (Sasaki-Sekimoto et al., 2005) and to assess multiple stress-responsive genes (Zheng et al., 2006). The cDNAs expressed under normal development conditions were immobilized on nylon membranes and hybridized with RNAs extracted from drought-stressed plants, as described in detail by Rodrigues et al. (2009, 2011). The level of drought tolerance of the cultivars employed in the gene expression study was considered from very low water deficit-sensitivity to high drought-resistance, based on productivity analyses carried out in field during dry seasons.

After monitoring the expression level of 3,575 leaves transcripts, the sugarcane plants SP83-5073 (the cultivar is considered highly drought tolerant) showed a smaller set of genes differentially expressed, most of them induced under severe water stress condition. Most of the expressed genes were related to polyamine synthesis, stress response and transport of water and solutes. The up-regulated transcript encodes an S-adenosylmethionine decarboxylase, a key enzyme in spermidine and spermine biosynthesis from putrescine. It is known that polyamines (putrescine, spermidine, spermine) are essential to plant growth by playing roles in cell division, tuber formation, root initiation, embryogenesis, flower development or fruit ripening (Crozier et al., 2000). Enzymes involved in polyamines biosynthesis have also been found under different abiotic stress conditions (Bouchereau et al., 1999). Although the polyamines role under these specific situations is not well understood yet, researches towards understanding its regulation under water stress (Alcázar et al., 2006) or low temperature (Cuevas et al., 2008) have been performed.

Other stress response proteins Bet v I allergen (a PR10 protein), Germin-like protein, Peroxidase or Disease resistance protein RPM1 (an R gene family protein) were also associated to the SP83-5073 responses to stress. The Bet v I allergen is a cytoplasmic disease resistance-related protein superfamily member which has been described in wounding events, high salinity conditions or cold stress (Radauer & Breiteneder, 2007), and the pathogenicity studies (Siemens et al., 2006). Similarly, a germin-like protein, member of large family of ubiquitous proteins involved in a wide range of plant metabolic processes as responses to stress was found (Vallelian-Bindschedler et al., 1998). Interestingly, germin-like proteins also seem to present a superoxide dismutase activity in some plants (Kukavica et al., 2005; Thornburg et al., 2003; Woo et al., 2000) and moss (Nakata et al., 2002). Enzymes of detoxification metabolism can be induced to scavenge reactive oxygen species, produced as consequence of the unbalanced metabolic reactions of a cell under water deficit conditions (Turkan et al., 2005). In addition, by detecting genes such as Bet v I allergen or germin-like protein or a RPM1 protein among differentially expressed genes of drought-stressed plants provides evidences that plants share mechanisms of acclimation not just to different abiotic stresses, but also to biotic stress.

Regarding genes involved in transport metabolism, different lipid transfer proteins as well as water channel proteins (ABC transporter and plasma membrane integral protein – PIP
protein) were induced by tolerant SP83-5073 plants in response to stress. Lipid transfer proteins contain chemical characteristics to bind and transport hydrophobic molecules and thus associated with enhanced cell wall extension in tobacco (Carvalho & Gomes, 2007; Nieuwland et al., 2009). It is supposed that these proteins induce the transfer of lipids through the extracellular matrix due to the increased accumulation of cuticular wax observed in tobacco leaves in response to drought events (Cameron et al., 2006). A water channel protein was also induced under mild stress in the tolerant cultivar SP83-2847. This protein called integral membrane protein TIP4-2 is an aquaporin present in tonoplast that plays a role in water transport across membranes, being related to adjusting the water status in response to environmental changes (Luu & Maurel, 2005).

Contrastingly, cultivar SP90-1638 (drought-sensitive) presented a distinct gene expression profile. A larger set of genes differentially expressed (induced or repressed due to water deficit) was observed under mild, moderate and severe stress. In the sensitive cultivar the number of genes increased as the stress became more severe. Based on the functional roles, one can observe that important stress-related genes involved in signal transduction, bioenergetics and photosynthesis were repressed. Changes in metabolism are needed for plants to protect themselves against stress. An earlier up-regulation in cell communication molecules serves as important messengers in transcriptional regulatory networks (Shinozaki & Yamaguchi-Shinozaki, 2000). An ineffective signal transduction cascade will probably result in inappropriate gene expression in response to stress. Sensitive plants did not show significant transducer genes being induced precociously under mild water deficit, or presented a down-regulation in moderate stress, but most of these genes were expressed just under severe conditions.

When plants are exposed to water deficit a decrease in photosynthetic rate is one of the first physiological changes that can be observed. In general this data is obtained through physiological analyses, measured by using specific parameters. As complementary information, genetic data assessed for the cultivar SP90-1638 showed that some genes involved in photosynthesis were up-regulated under mild water deficit conditions. However, under moderate and severe stress several genes (transcripts codifying for photosystem proteins, plastocyanin precursor, thioredoxin M-type, oxygen-evolving enhancer protein 2, and even a protein involved in the absorption and transfer of energy between photosystems) related to this metabolism were down-regulated. At the first signs of water deficit, plants close the stomata to avoid excessive water loss by transpiration (Rachmilevitch et al., 2006). As a consequence under moderate stress photosynthesis becomes affected and eventually is inhibited by increased water stress severity (Taiz & Zeiger, 2006). Transcripts encoding a ferredoxin I chloroplast precursor, a plastocyanin precursor and a photosystem I complex PsaN subunit precursor were induced under mild stress condition and subsequently repressed under moderate and severe stress, indicating that in this plants the water stress imposed was limiting to the photosynthesis process. The gene expression pattern identified for this metabolism corroborates to physiological conditions and could be used as an indicative of drought sensitivity for this Brazilian sugarcane cultivar. In plants where the loss of water relative content was small, the photochemical activity was less affected by water stress, supporting the concept of plant productivity loss under drought conditions (Liu et al., 2006).

On the other hand, in the tolerant sugarcane cultivar SP83-2847 the photosynthetic rate decreased under mild stress, but several genes related to photosynthesis were induced under this condition, probably because the stress was not strong enough to produce
photoinhibition. According to Silva et al. (2007) water deficit can severely reduce the productivity of sugarcane because photosynthetic rate decreases progressively as stress becomes more severe (Bhatt et al., 2009; Bloch et al., 2006; Dulai et al., 2006). However, genes involved in photosynthesis process were found to be up-regulated. It is suggested that water stress was enough to trigger plants physiological responses to protect them against the stress, but it was not so severe as to repress the expression of the photosynthesis genes. Better adapted plants are also more efficient in water use and show greater tolerance to drought-stress (Munns 2002; Xu & Hsiao 2004).

The stress response metabolism in sensitive plants was more down-regulated in contrast to those verified in tolerant plants SP83-5073. Despite few genes having been differentially expressed in tolerant plants, 94% of them were up-regulated by stress, whereas 45% of the genes expressed in sensitive plants were down-regulated under water stress conditions. An antagonistic pattern was verified for some genes which were induced in tolerant cultivar SP83-5073 and appear to be repressed in sensitive cultivars. Metabolism induced by SP83-5073 plants such as lipid metabolism (also induced by tolerant plants SP83-2847) or polyamines biosynthesis, appeared down-regulated in sensitive cultivar SP90-1638. Lipid metabolism, including significant proteins (chloroplast phytoene synthase 1, very-long-chain fatty acid condensing enzyme CUT1, esterase, lipase, phospholipase D, and others) were also repressed in sensitive plants. Among biochemical pathways, phospholipase D plays an important part in phosphatidic acid generation from phosphatidylcholine breakdown. Besides, this enzyme had been related to biotic and abiotic stresses as a signal transducer (Bargmann & Munnik, 2006; Zhang et al., 2005), the involvement of the phospholipase D in glycinebetaine biosynthesis have been proposed (Bray, 2002).

The global transcriptome analysis of cultivar SP83-2847, ranked as moderately tolerant, showed a large amount of genes being induced or repressed after plants were exposed to mild, moderate and severe water deficit (Rodrigues et al., 2011). An enzyme involved in ABA synthesis, as well as an ABA-regulated protein presented, during the whole period under stress, a high induction level. Endogenous ABA content increases as stress becomes more severe to protect plants either playing roles in physiological behavior or by regulating gene expression. In ABA-dependent pathways, genes have an ABA-responsive element (ABRE) with affinity for MYB and bZIP transcription factors that signal for expression of specific genes involved in plant stress response. The transcription factors DREB act on dehydration-responsive cis-acting element (DRE) to trigger gene expression in an ABA-independent pathway (Shinozaki & Yamaguchi-Shinozaki 2007; Agarwal & Iha 2010). Transcription factors expressed by SP83-2847 plants indicate that ABA-dependent and ABA-independent pathways are presented in sugarcane responses to water deficit. Genes observed in this class included some proteins such as NAC1, DREB1, bZIP, MYB, MYC, among others. Once activated, transcription factors act as DNA-binding proteins, which are capable of mediating the transcription of key proteins in the stress response mechanism. Overall, tolerant plants induce genes under severe water deficit (Rodrigues et al., 2009) or trigger the main expression over the time under stress. It is a fact that some plants can tolerate stress events more efficiently than others. The Festuca mairei is a grass that can be used as reference in genomic studies to be compared to other grass due to its genetic adaptation to drought (Wang & Bughrara, 2007). Most of the genes repressed under drought stress in this grass are related to biogenesis and cellular metabolism whereas the induced genes are involved with transcription and defense (Wang & Bughrara, 2007). In addition, in our gene expression experiments a large number of unknown genes were determined;
which may represent a source of new variability in water deficit tolerance studies. For instance in SP83-2847 a large set of genes was differentially expressed under water deficit conditions, however, genes similar to unknown or those with no similarity in databases represent approximately 76% of the genes expressed by these tolerant plants.

The characterization of the gene expression profiles under stress is an important tool for plant breeding and understanding the genetic basis of drought tolerance becomes an essential knowledge in this scenario. The development of drought-tolerant plants is an alternative for areas with restricted water availability (Cushman & Bohnert, 2000). In this context, molecular techniques are a powerful tool to identify genes involved in plant responses and it also allows manipulating only a specific characteristic. The macro and microarray technology applied to the identification and characterization of genes involved in drought tolerance has brought knowledge about the perception of the stress and how plants respond to this adverse condition.

5. Genetic improvement and perspectives

Sugarcane has gone through a fantastic transformation from a wild canes with thick and not so juicy culms to commercial cultivars with thicker and juicier stalks in the hands of plant breeders who used crosses and selection processes aiming to improve sugarcane yield, disease resistance, biomass yield and sucrose content, and is now a specially important crop in several tropical countries. It is known that creating a new cultivar takes approximately 15 years. Traditional breeding can use biotechnological tools as molecular markers in the selection process for better genotypes but, even using the biotechnology to generate new improved cultivars a big effort has to be inputted by breeders in order to assure they have enhanced their economic agronomic traits to meet the energy and food demand respecting land use.

In plant breeding programs, the molecular markers technique has been used to select cultivars thereby reducing the time between selection of materials and commercialization of improved cultivars. In addition, physiological parameters studies also provide the selection of cultivars by comparing well characterized genotypes in relation to drought tolerance. Even though the sugarcane genome study has not been finished there are thousands of ESTs that have been generated over the last decade and are helping researchers around the world understand metabolic pathways and plant responses to environmental changes.

Recently Gornall, et al., (2010), published a review on all the implications of climate changes on agriculture productivity for the twenty first century and how it will impact agriculture around the world, as we know agriculture is strongly influenced by climate changes. Global warming may cause an increase in heat stress and water evaporation. Most of the plantations are rain-fed and changes in precipitation patterns will enormously affect crop production and current cultivars will reduce yield. Food supplies and energy demand are very important issues for the world growing populations.

Not only for sugarcane but also considering other potential crops as soybeans, corn or sugar beet, that are being considered for biofuel production, the equation of food supply and energy production has been a great challenge for most economic specialists in many countries. According to projections of global warming, the changes in climate can worsen the negative effects of water deficit upon agriculture. As drought is a climate factor inherent to all plants, sugarcane crops yields might decrease significantly, emphasizing the need for adapted cultivars. In this matter understanding the gene expression pattern of tolerant and
sensitive plants can provide other tools to maximize the selection and development of new cultivars. Thus, the improvement of crops is essential to support longer periods of drought and it is crucial to maintain and expand the crop yield considering the future demand for food and competitiveness of the biofuel and ethanol business.

The development of new cultivars tolerant to drought along with other characteristics such as poor soil is essential to expand sugarcane plantation. Despite the genetic complexity of sugarcane, plant transformation has been developed in genetic improvement programs. A sugarcane transcriptional regulator of the ethylene responsive factor (ERF) superfamily was expressed in tobacco in response to drought and salt stress (Trujillo et al., 2008). Sugarcane also accumulated a significant amount of sucrose in immature tissues after been genetically engineered to repress a gene involved negatively in bioenergetics metabolism (Groenewald & Botha, 2008).

In Brazil the success of sugar and ethanol production is a result of increases in crop and industry productivity. The country has several breeding programs that frequently release new improved varieties. Cultivars resistant to drought, pests and herbicide tolerant plants should be released in the next few years thanks to the advances generated by the molecular understanding of metabolic pathways such as those involved in the water stress. This scenario was enforced by efforts from FAPESP, the state of São Paulo funding agency, which in the late 1990s established SUCEST, an integrative program to study sugarcane transcriptome. Later biotech companies Alellyx and Canaviallis, now bought by Monsanto, were created and devoted time to integrating all their knowledge for the development of new sugarcane varieties. Sugarcane industry used in the last centuries to produce sugar and later alcohol, has became now a diversified industry focusing on different sectors such as chemical, cosmetics and bioplast production. These new perspectives are a great opportunity for Brazil and other countries in the Tropics.

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Web Site Reference
Sucest project: http://sucest.lbi.ic.unicamp.br/en
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Plants experience water stress either when the water supply to their roots becomes limiting, or when the transpiration rate becomes intense. Water stress is primarily caused by a water deficit, such as a drought or high soil salinity. Each year, water stress on arable plants in different parts of the world disrupts agriculture and food supply with the final consequence: famine. Hence, the ability to withstand such stress is of immense economic importance. Plants try to adapt to the stress conditions with an array of biochemical and physiological interventions. This multi-authored edited compilation puts forth an all-inclusive picture on the mechanism and adaptation aspects of water stress. The prime objective of the book is to deliver a thoughtful mixture of viewpoints which will be useful to workers in all areas of plant sciences. We trust that the material covered in this book will be valuable in building strategies to counter water stress in plants.

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