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Transcriptomics of Sugarcane Osmoprotectants Under Drought

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1. Introduction

Sugarcane (*Saccharum* spp.) is an alogamous plant from the Poaceae family and the Andropogoneae tribe (Daniels & Roach, 1987). This crop covers more than 23 million hectares worldwide, representing about 0.5 % of the total global area used for agriculture, with a production of 1.6 billion metric tons of crushable stems (FAOSTAT, 2009). Brazil is the world's largest producer, contributing with two-thirds of total sugar production - about 31 million tons per year - of which 19.5 million tons are exported (UNICA, 2009). Sugarcane, its derivatives and by products have received great attention, due to their multiple uses, with emphasis on the ethanol production, representing an important renewable biofuel source. It has been estimated that sugarcane ethanol fuel may replace up to 10.0 % of the world's refined petroleum products consumption in the next 15 to 20 years (Goldemberg, 2007). Despite its importance and similarity to other important agronomic crops, the sugarcane production has been adversely influenced by many environmental factors such as harsh climate and soil conditions.

Abiotic stresses are among the main causes of losses in the productivity of the major crops worldwide (Bray *et al.*, 2000), a scenario where drought figures as the most significant stress, causing negative impacts on crop adaptation and productivity. Besides, this condition can exacerbate the effect of other stresses (biotic or abiotic) to which the plants may be submitted. Although breeding activities have provided significant progress for the understanding of the physiological and molecular responses of plants to water deficit, there is still a large gap between yields in optimal and stressful conditions (Cattivelli *et al.*, 2008). Essays regarding plant responses to drought stress have been published applying technologies of functional genomics (Wang *et al.*, 2011). These evaluations provided important insights into molecular and biochemical mechanisms in the study of drought tolerance in various crops and model species. Plants are able to ''perceive'' the external stimuli by multiple sensors, recognizing adverse situations and invoking signal transduction cascades and consequently secondary messengers, activating stress responsive genes (Grennan, 2006), resulting in both molecular and physiological responses. Among the mechanisms developed by plants to face the adverse conditions generated under drought, the accumulation of osmoprotectants compounds are often recognized as a mitigation mechanism of the negative consequences of water deficit (Choluj *et al.*, 2008).

Osmoprotectants are small solutes used by cells of numerous water-stressed organisms and tissues to maintain cell volume (Yancey, 2001), and may play other roles regarding tolerance, as proteins stabilizing and antioxidant action (Rathinasabapathi, 2000). They include sugars, mainly fructose and sucrose, sugar alcohols (like myo-inositol), complex sugars (like trehalose and fructans) and charged metabolites (like glycinebetaine, proline and ectoine) (Yancey, 2005).

Previous information regarding sugarcane osmoprotectants under stress was reported. For example Suriyan & Chalermpol (2009) analyzed diverse parameters in sugarcane submitted to iso-osmotic salt and water-deficit stresses. Among the physiological alterations, an increase in the proline content in stressed-leaves was positively correlated to the reported stresses, indicating a key role of proline in osmoregulation and antioxidant defense mechanisms. Also Rasheed et al. (2010) investigated the possible roles of proline and glycine betaine (GB) in mitigating the effect of chilling stress in the sprouting nodal buds of sugarcane. To accomplish this evaluation, they performed a pre-treatment with proline bud chips and GB, obtaining a substantial reduction in the H_2O_2 production and an increase in the synthesis efficiency of soluble sugars, protecting developing tissues from the effects of chilling stress. Recent molecular research works, regarding drought and salinity performance in sugarcane, were carried out using techniques based on molecular hybridization such as SSH [Subtractive Hybridization Suppressive] (Patade et al., 2010) and micro/macroarrays (Rodrigues et al., 2009). In a general view, the main limitations of these methods regard their low sensibility and specificity (Shimkets, 2004). Moreover, in relation to micro/macroarrays, in spite of their high performance and broad use, the inability to analyze and discover new genes have been reported (Wang et al., 2009). Techniques based on sequencing [i.e. SAGE (Velculescu et al., 1995) and derivatives] take advantage of the available frequencies of fragments (tags) representing transcripts expressed in the sample, by the assumption that a short and defined tag contains the information needed to identify the corresponding cDNA (Velculescu et al., 1995). Thus, besides constituting an open architecture analysis (i.e., allowing the discovery of new genes), the abundance of tags found for a given gene provides an estimative of their transcription in the sample. In this context, the SuperSAGE technique (Matsumura et al., 2003) stands out for its efficiency in generating transcription profiles, especially with the actual association to the high performance sequencing platforms [Pyrosequencer (454 Roche®), Solexa (Illumina®) and SOLiD (Applied Biosystems®)].

The SuperSAGE method is characterized by the generation of 26 bp tags by using the type III restriction enzyme EcoP15I (Matsumura et al., 2003). This technique presents itself as one of the most modern tools of functional genomics and provides some advantages such as improvements in tag-to-gene annotation, simultaneous analysis of two interacting eukaryotic organisms, full-length cDNAs amplification using tags as primers, potential use of tags via RNA interference (RNAi) in gene function studies, identification of antisense and rare transcripts, and identification of transcripts with alternative splicing (Matsumura et al., 2006). It also provides a global and quantitative transcriptomic analysis based on the study of the entire transcriptome produced in a given time under a given stimulus. This technique has been successfully applied in plant species such as rice (Matsumura et al., 2003), banana (Coemans et al., 2005), chili pepper (Hamada et al., 2008), chickpea (Molina et al., 2008; 2011), tobacco (Gilardoni et al., 2010) and tropical crops (cowpea, soybean, sugarcane; Kido et al., 2010). Some of them using the association of SuperSAGE with a high-throughput sequencing platform (HT-SuperSAGE; Matsumura et al., 2010). In the present work we

profit from the high resolution power of SuperSAGE® coupled to the Illumina® sequencing in a SuperTag Digital Gene Expression (STDGE) profile (GenXPro GmbH, Frankfurt, Germany) trying to characterize the transcriptome of drought-stressed sugarcane roots after 24 hours of submission to this stress, aiming to elect a best group of tags to be validated by RTqPCR. For this purpose, a high-throughput transcriptome project as a joint Brazilian initiative from UFPE (Federal University of Pernambuco) and CTC (Sugarcane Technology Center) was carried out. The project generated a large amount of gene candidates from different important categories considering the response against this kind of stress. In the present chapter an overview regarding the identification, categorization and differential expression of osmoprotectants will be evaluated in sugarcane, compared with the up to date knowledge concerning this crop and related species. Considering its role in world's economy and biotechnological potential, the identification and expression profile of responsive osmoprotectant coding genes in sugarcane may be helpful to unravel the basic mechanisms of stress tolerance, bringing valuable evidences for sugarcane improvement.

2. Drought: Understanding the problem

Biological stress may be defined as an adverse environmental condition that inhibits the normal operation of a biological system such as plants (Jones & Jones, 1989). The life in the terrestrial condition represents a challenge to plants, and often have to occupy environments that are not the most appropriated for their development, being also subjected to frequent environmental changes in their native conditions. A large number of abiotic factors associated with plant-water relations – such as drought, salinity, chilling, frost and flooding – negatively affect the overall growth of terrestrial plants, leading to stunted form, metabolic changes, reduced yields, germination problems and even plant death under extreme conditions (Smith and Bhavel, 2007). Among the mentioned factors, drought stands out, bringing the most serious threat in view of the existing freshwater shortage in many regions of the world, bringing serious limitations to the agriculture (Jury & Vaux, 2005).

Notwithstanding the availability of more than 150 definitions for drought in the literature (Boken, 2005), it is often defined in terms of available humidity as compared with a normal value, with the severity correlating in function to the time and magnitude of the exposition to a deficient humidity (Smith & Pethley, 2009). Among the diverse types reported, some can be highlighted as meteorological, hydrological, socioeconomic and agricultural drought (Boken, 2005; Smith & Pethley, 2009). According to Boken (2005), the meteorological drought occurs when seasonal or annual precipitation falls below its long-term average; the hydrological drought when the meteorological drought is prolonged and causes local shortage of surface and groundwater; the socioeconomic drought is a manifestation of continued drought of severe intensity that shatters the economy and sociopolitical situation in a region, while the agricultural drought sets, due to soil moisture stress, a significant decline in crop yields (production per unit area).

The agricultural drought is the most important nowadays and will be here focused. Agriculture is by far the largest consumer of water, representing for 80.0 % of the freshwater consumption worldwide (Jury & Vaux, 2005). Economic losses associated to water availability reached about one billion dollars, in 2009, only in the United States (Anderson *et al.*, 2009). Actually, one-third of the world population lives in areas with water shortages. This is specially serious considering other adverse factors such as the high levels of

atmospheric CO_2, climate change scenarios and predictions of future global warming, all of them increasing drought incidence, frequency and severity. Significant further problems are predicted for food production due to the limited availability of suitable freshwater for agriculture.

According to Hazell & Wood (2008), the climate change will affect different localities in different ways, with potential benefits to some important food growing areas as the Canadian Prairies, but making agriculture more difficult in some other regions as many drought prone areas in Africa and Americas. Such predictions also reinforce that agricultural systems have considerable capacity to adapt to climate change, but this poses many challenges that are not yet fully understood, with urgent research efforts necessary to identify the best ways to adapt.

3. Sugarcane and drought: Current outlook and use of science in search of solutions

Sugarcane is one of the world's major food crops, providing about 75.0 % of the sugar harvested for human consumption [Food and Agriculture Organization (FAO) statistics]. The crop is closely associated with sustainability as it is presented as a renewable energy source including ethanol and electricity (Tew & Cobill, 2008). Despite this status, sugarcane suffers severe losses due to the availability of soil water. In Brazil, the largest global sugarcane producer (Henry, 2010), the state of São Paulo accounts for about 60.0 % of the country production, suffering losses of around 10.0 % due to periods of drought, as reported for harvests in 2010 (UNICA, 2011). Thus, it is necessary to generate new tolerant varieties to this stress. Currently, breeding programs for sugarcane are being developed in different countries by public and private institutions, and also by cooperative systems formed by producers. Most projects involve the performance of controlled crossings which are costly and bring long term results, reaching sometimes more than 15 years (Cesnik & Miocque, 2004).

An alternative way to promote the breeding is associated with the analysis and identification of specific genes involved in given metabolic processes. The traditional method for identifying a gene responsible for a particular trait includes the initial demonstration that the trait is inheritable, followed by isolation of a candidate gene that is postulated to be responsible for that trait. This "single gene" approach is fundamentally flawed for many traits since it is assumed that every trait is governed by a single gene. According to Casu *et al.* (2010) genomics and all of the related "omics" techniques, e.g. transcriptomics, break this formula and rely on the in-depth assembly of large amounts of data followed by data-mining to determine connections between a particular trait and any number of associated genes.

Transcriptomics data are available for sugarcane, and have been generated mainly by EST (Expressed Sequence Tag) sequencing, or using methodologies based on probe hybridization arrays, using known genes from other crops. Among the available collections of ESTs for sugarcane, the sequences generated by the SUCEST project should be highlighted. This project regards a large consortium of Brazilian researchers who sequenced approximately 238,000 redundant ESTs from 26 diverse cDNA libraries (Vettore *et al.*, 2001), representing the largest effort to generate information using new technologies for this species. This endeavor brought a broader panel when compared to previous available ESTs produced by other consortia in countries like Australia (Casu *et al.*, 2003, 2004; Bower *et al.*, 2005) and the US (Ma *et al.*, 2004).

Additionally, microarray platforms have been also used to evaluate sugarcane expression profile. The first ones were used to investigate gene expression differences between immature and maturing stems of sugarcane (Casu *et al.*, 2003; 2004). Using glass microarrays the authors assayed up to 4,715 non-redundant random ESTs derived from immature and maturing stems, and also from roots. The most recent report using this method made use of a custom cDNA microarray (3,598) to profile the effect of elevated CO_2 on sugarcane leaves (De Souza *et al.*, 2008). Regarding open architecture transcriptomics technologies – which analyze the entire population of transcripts produced in a given time under a given stimulus – a single literature report is available for sugarcane, in an approach developed by Calsa & Figueira (2007). The authors used standard 14 bp SAGE to characterize the sugarcane mature leaf transcriptome, generating 9,482 valid tags, with 5,227 unique sequences, from which 3,659 (70.0 %) matched at least one sugarcane assembled sequence with putative function.

Despite the relative data abundance for sugarcane transcriptome covering different conditions, there is still a restricted number of publications regarding the analysis of molecular behavior under drought, whilst most available evidences come from technologies based on hybridization or cDNA (EST) sequencing. Rocha *et al.* (2007) in a cDNA microarrays approach to profile expression of 1,545 genes involved in signaling processes in plants submitted to drought, phosphate starvation, herbivory and N_2-fixing endophytic bacteria, identified 485 differentially expressed candidates after exposition of the plants to water shortage. In another approach Gupta *et al.* (2010) using cDNA libraries associated to the RTqPCR validation of drought related genes, revealed differences greater than 2-fold regarding the expression of given genes during dehydration stress. The most recent report was carried out by Iskandar *et al.* (2011) that investigated whether the degree of expression of eight stress-related genes - *P5CS, OAT, AS, PST5, TF1, LEA, POX* and *dehydrin* – was correlated with the sucrose content in the sugarcane culm, and whether such genes were also responsive to water deficit stress. Almost all selected genes were upregulated, with exception of *POX* that was downregulated after 15 days of water deficit stress. However, subsequent analysis revealed a different transcriptional profile to that, showing a correlation with the sucrose accumulation. For example, genes with homology to late embryogenesis abundant-related proteins and dehydrin were strongly induced under water deficit, but did not correlate with sucrose content. The expression of genes encoding proline biosynthesis was associated with both sucrose accumulation and water deficit, but amino acid analysis indicated that proline was negatively correlated with sucrose concentration.

Since drought is a complex feature discovery of genes associated to tolerance processes is urgent, being one of the most important and difficult challenges. A larger number of studies is made necessary, aiming to understand this issue in sugarcane, enriching the knowledge on the metabolic pathways involved in acclimation process to water deficit.

4. Drought and osmoprotectants in plants

Water deficit, caused by "lack of water" or by other environmental stresses like extreme temperatures or salinity (Bartels & Souer, 2004), has been great problems for agriculture, affecting virtually every aspect of plant physiology and metabolism. As a consequence of these stresses, a range of adaptive responses including morphological (Jaleel *et al.*, 2009), physiological (Harb *et al.*, 2010) and biochemical changes (Ahmadi *et al.*, 2010) enabled plants to tolerate and survive at such adverse conditions. Similar cellular and molecular adaptive responses include a significant accumulation of compatible solutes.

Compatible solutes regard a variety of low-molecular-weight organic compounds, electrically neutral molecules, soluble in water and nontoxic at high cellular concentrations (Yancey, 2001). Such osmolytes include a variety of simple sugars (e.g. fructose and glucose), sugar alcohols (glycerol and methylated inositols) and complex sugars (trehalose, raffinose and fructans), while other include quaternary amino acid derivatives (proline, glycine betaine, b-alanine betaine, proline betaine), tertiary amines (ectoine; 1,4,5,6-tetrahydro-2-methyl-4-carboxy-lpyrimidine) and sulfonium compounds (choline o-sulfate, dimethyl sulfonium propironate) (Rhodes & Hanson, 1993; Vinocur & Altman, 2005). Compatible solute accumulation in response to osmotic stress is a ubiquitous process in organisms as diverse as bacteria, plants and animals (Bohnert & Jensen, 1996). These osmoprotectants compounds are typically confined mainly to the cytosol, chloroplasts, and other cytoplasmic compartments (Rontein *et al.*, 2002), protecting plants in different ways, including: stress defense by osmotic adjustment (helping the cells to maintain their hydrated state and turgor maintenance), stabilization of proteins and enzymes, induction of stress proteins and acceleration of reactive oxygen species scavenging systems (Bohnert & Jensen 1996; Ashraf & Foolad, 2007). In plants that naturally accumulate osmoprotectants, the level of these compounds are highest under stress extension (Rhodes & Hanson, 1993). So, changes in plant drought-induced gene expressions have been revealed, and many genes have been isolated from numerous species, playing important roles in both initial stress response and in establishing plant stress tolerance (Shinozaki & Yamaguchi-Shinozaki, 2007). Proline, glycine betaine, sugars and sugar alcohols are examples of compatible solutes encoded by some of these stress-inducible genes that function in cellular osmotic adjustment, promoting drought tolerance, guaranteeing plasma membrane integrity, without disrupting the protein function (Bartels & Sunkar, 2005). So, the osmotic adjustment by accumulation of these compounds has been proposed as an important mechanism to overcome the negative consequences of water deficit in crop production (Rathinasabapathi, 2000; Choluj *et al.*, 2008). Based on accumulation of these compounds to be associated to high levels of tolerance in plants, and considering that their beneficial effects are generally not species-specific (Rontein *et al.*, 2002), considerable progress has been achieved in investigations using transgenic plants overexpressing selected osmoprotectants conferring abiotic stress tolerance (Ashraf & Foolad, 2007; Chen & Murata, 2008). Some of them are reviewed below.

4.1 Glycine betaine

Glycine betaine (GB) is a quaternary ammonium compound (QAC) synthesized by a great variety of organisms, including plants, animals and microorganisms (Rhodes & Hanson, 1993). In most organisms GB is synthesized either by the oxidation (or dehydrogenation) of choline or by the N-methylation of glycine. However, the pathway from choline to glycine betaine has been the main GB-accumulation pathway in plant species (Weretilnyk *et al.*, 1989). In this pathway choline is converted to betaine aldehyde by choline monooxygenase (CMO) (Rathinasabapathi *et al.*, 1997), which is then converted to GB by betaine aldehyde dehydrogenase (BADH) (Vojtechova *et al.*, 1997). Similarly to proline (and other osmoprotectants in plants) GB is one of the most extensively studied compatible solutes, being upregulated after drought (Ma *et al.*, 2007), salinity (Kern & Dyer, 2004), low temperature (Zhang *et al.*, 2010) and oxidative stresses (Liu *et al.*, 2011). *In vitro* assays indicate that GB acts as an osmoprotector, stabilizing both the quaternary structure of proteins and the highly ordered membrane structure under adverse conditions (Gorham,

1995). Based on the correlation between GB accumulation and stress tolerance, progress in exogenous GB application (Jokinen *et al.*, 1999), cloning and expression of GB encoding enzymes has been achieved (Sakamoto & Murata, 2002; Quan *et al.*, 2004). Examples of these genes include *CMO* (Tabuchi *et al.*, 2005), *BADH* (Wood *et al.*, 1996); *CDH* and *BADH* (Landfald & Strøm, 1986), which were cloned from different organisms and introduced into transgenic plants. For example, in transgenic cotton (transformed with *bet*A gene) GB expression induced the protection of the cell membrane integrity from drought stress damage, being also active in osmotic adjustment (Lv *et al.*, 2007). Huang *et al.* (2000) transformed three different species (*Arabidopsis thaliana*, *Brassica napus* and *Nicotiana tabacum*) with the *COX* gene from *Arthrobacter pascens*, an elucidative approach considering that these plants are non accumulators of this osmoprotector. The highest levels of betaine in independent transgenic plants were de 10- to 20-fold lower than the levels found in natural betaine producers. Further, it was observed that the supplementation of choline is necessary to allow the accumulation of physiologically relevant amounts of betaine. Despite of that, the authors reported the acquisition of a moderate stress tolerance (drought and salinity) in some but not all betaine-producing transgenic lines, based on the relative shoot growth; while the responses to salinity, drought, and freezing stresses were variable among the three transformed species. The results lead to the supposition that higher efficiencies would be achieved in species that naturally produce this osmoprotector (Huang *et al.*, 2000). Similar results were achieved by Shirasawa *et al.* (2006) after transformation of rice plants (*Oryza sativa*) – also a non accumulator of GB - with the choline monooxygenase gene from spinach. Enhanced tolerance to salt stress and temperature stress in the seedling stage was observed, however the CMO-expressing rice plants were not effective for accumulation of GB and improvement of productivity. Considering sugarcane, Patade *et al.* (2008) observed the accumulation of free proline and glycine betaine in embryogenic sugarcane calli *(Saccharum officinarum* L.; cv. CoC-671) after NaCl stress. The gradual increase in glycine betaine was positively correlated with the concentrations of NaCl (up to 213.9 mM). Indeed, such osmoprotector was also higher as compared to proline content, in all stress conditions tested (NaCl treatments: 42.8 to 256.7 mM). However, in the higher NaCl concentration, proline was not observed.

4.2 Proline

Proline (Pro) is a proteinogenic amino acid essential for primary metabolism. It is considered one of the most important osmolytes, being accumulated in a large number of species in response to stress damage (for a review, see Hare & Cress, 1997). Under abiotic stress condition, proline accumulation is involved in the maintenance of turgor, promoting continued growth in case of low water potential in the soil (Mullet & Whitsitt, 1996). The accumulation of this important osmolyte, upon osmotic stress, is well documented in a large number of different organisms, including, protozoa (Poulin *et al.*, 1987), eubacteria (Csonka, 1989) and marine invertebrates (Burton, 1991). In addition to its role in the osmotic adjustment mechanism, other important functions have been attributed to proline (Bartels & Sunkar, 2005) such as protection of plasma membrane integrity, enhancing of different enzymes activity (Sharma & Dubey, 2005; Mishra & Dubey, 2006), regulation of nitrogen and carbon reservoir (Kishor *et al.*, 2005) and as scavenger of free radicals (Smirnoff & Cumbes, 1989).

In higher plants, proline biosynthesis may proceed from two different ways: either via glutamate, by successive reductions catalyzed by pyrroline-5-carboxylate synthase (P5CS) and pyrroline-5-carboxylate reductase (P5CR), respectively (Hu *et al.*, 1992; Savouré *et al.*,

1995) or ornithine pathway, by ornithine d-aminotransferase (OAT) (Mestichelli *et al.*, 1979). Here the first pathway will be discussed, since it is considered the main pathway during osmotic stress in plants (Bartels & Sunkar, 2005; Parida *et al.*, 2008) especially considering the drought response. Under water deficit, proline is synthesized from the glutamate by two intermediates. In the first step, the glutamate is reduced to glutamic acid-5 semialdehyde (GSA) by P5CS. The GSA produced is converted into pyrroline-5-carboxylate (P5C) (Hu *et al.*, 1992; Savouré *et al.*, 1995) which is then reduced by P5CR to proline (Zhang *et al.*, 1995).

Proline induction in response to abiotic stresses has been related for many angiosperms (Mohammadkhani & Heidari, 2008; Székely *et al.*, 2008), revealing a positive relationship between proline accumulation and stress tolerance in this group. Kishor *et al.* (1995) reported the overexpression of a *Vigna aconitifolia P5CS1* gene in tobacco plants, leading to increased levels of proline (10- to 18-fold when compared to the control plants), enhancing root biomass, growth rhythm and tolerance under drought-stress. The importance of proline metabolism in the process of drought tolerance was evidenced by Ronde *et al.* (2000) in soybean plants (*Glycine max*). The authors reported the suppression of proline synthesis in transgenic soybean plants containing the *P5CS* gene in the antisense direction. Transformed plants presented increased sensitivity to water deficit, as compared with the wild type. In cotton under drought-stress, Parida *et al.* (2008) verified an induction of proline levels by the upregulation of P5CS and downregulation of proline dehydrogenase (PDH), indicating a possible involvement of proline production in the development of drought tolerance. Osmotic adjustment through proline accumulation was reported as a primary response of drought stressed sugarcane (*S. officinarum*) plantlets (Errabii *et al.*, 2006).

On the other hand, reports suggested that the increase in proline concentration is related to protective symptoms under severe water stress rather than an osmoregulatory function. In transgenic wheat plants, the higher accumulation of proline (when compared to the wild type) conferred drought stress tolerance by increasing the antioxidant metabolism rather than increasing osmotic adjustment (Vendruscolo *et al.*, 2007). In sugarcane transformed with the *V. aconitifolia P5CS* gene, it was observed that after nine days without irrigation proline content in transgenic plants was on the average 2.5-fold higher than in the controls. However, no osmotic adjustment was observed in plants overproducing proline during the water-deficit period, suggesting a role of proline as component of the antioxidative response system rather than as a promoter of osmotic adjustment (Molinari *et al.*, 2007). Indeed, the hypothesis of the protective role played by proline under severe drought stress was also supported by Gomes *et al.* (2010), who evaluated the water stress effect on osmotic potential, proline accumulation and cell membrane stability in leaflets of the coconut palm (*Cocos nucifera* L.).

4.3 Myo-inositol

Inositol is a cyclohexanehexol, a cyclic carbohydrate with six hydroxyl groups, one on each carbon ring. Among the nine types of existing steroisomers, myo-inositol is the most abundant in the nature, being also important for the biosynthesis of a wide variety of compounds including inositol phosphates, glycosylphosphatidylinositols, phosphatidylinositides, inositol esters, and ethers in plants (Murthy, 2006). Besides the own myo-inositol, other related or derived molecules are also important osmoprotectors. Myo-inositol serves as a substrate for the formation of galactinol, the galactosyl-donor that plays a key role in the formation of raffinose family oligosaccharides (RFOs, raffinose, stachyose, verbascose) from sucrose. RFOs accumulate in plants under different stress conditions

(Kaplan *et al.*, 2004; Peters *et al.*, 2007). In the case of the halophyte *Mesembryanthemum crystallinum* (common ice plant) – that possesses a remarkable tolerance against drought, high salinity, and cold stress – inositol is methylated to D-ononitol and subsequently epimerized to D-pinitol. This plant accumulates a large amount of these inositol derivatives during the stress (Adams *et al.*, 1992; Vernon *et al.*, 1993).

Throughout the biological kingdom, myo-inositol is synthesized by a two-step pathway that is unofficially known as the "Loewus pathway". The first step is the conversion of D-glucose-6-P to D-myo-inositol (1)-Monophosphate, 1D-MI-1-P, which is catalyzed by an L-myo-inositol 1-phosphate synthase (MIPS) (Majumder *et al.*, 1997), followed by its specific dephosphorylation to free myo-inositol by the Mg^{++} dependent L-Myo-inositol 1-phosphate phosphatase (IMP) (Parthasarathy *et al.*, 1994). Due to the potential of myo-inositol, some transgenic plants expressing this substance have been generated, mainly using MIPS enzyme or inositol derived enzymes.

Majee *et al.* (2004) reported on the isolation of the *PINO1* gene (also known as *PcINO1*, encoding an l-myo-inositol 1-phosphate synthase) from the wild halophytic rice relative *Porteresia coarctata*. This gene was expressed in tobacco plants, conferring them the capacity of growth in 200–300 mM NaCl with retention of ∼ 40–80 % of the photosynthetic competence with concomitant increased inositol production when compared with unstressed control. Additionally, *PINO1* transgenics showed *in vitro* salt-tolerance, confirming *in planta* functional expression of this gene.

Das-Chatterjee *et al.* (2006) carried out a functional introgression of *PcINO1* and *OsINO1* genes (this last regarding the corresponding homologue from the cultivated rice that encodes for a salt-sensitive MIPS protein) in distantly related organisms, as prokaryotes (*Escherichia coli*) to eukaryotes (yeast: *Schizosaccharomyces pombe*; plants: *Oryza sativa* and *Brassica juncea*) analyzing the tolerance of these transgenic lines under salinity stress. The results confirmed the role of the *PcINO1* gene, conferring salt tolerance to various levels of complexity, from prokaryotes to different eukaryotes, including higher plants, leading to an unabated production of inositol and survival under NaCl stress. Patra *et al.* (2010), in turn, held introgression and functional expression studies in tobacco plants using *PcINO1* (a) and *McIMTI* (b) [inositol methyl transferase, IMTI, from the common ice plant *M. crystallinum*] genes. After submission of the obtained transgenic lines to saline and oxidative stresses it was observed that all plants presented higher performances in terms of growth potential and photosynthetic activity and were less prone to oxidative and salt stresses when compared to the controls. Physiological experiments demonstrated the superiority of the *PcINO1–McIMT1* double transgenic plants to withstand the salt stress accompanied by the accumulation of both myo-inositol and methylated inositols in the system over the transgenic plants with either of the single gene(s).

4.4 Trehalose

Trehalose is a non-reducing α,α-1,1-linked glucose disaccharide that functions as an energy source and a storage form of more reactive glucose in lower organisms (Galinski, 1993). At least three different pathways for the biological synthesis of trehalose have been reported (Elbein *et al.*, 2003). In plants, the synthesis of this sugar occurs normally by the formation of the trehalose-6-phosphate (T6P) from the UDP-glucose and glucose-6-phosphate, a reaction catalyzed by the trehalose 6-phosphate synthase (TPS). Afterwards the T6P is dephosphorylated by the trehalose-6-phosphate phosphatase (TPP) resulting in the formation of free trehalose (Wingler, 2002).

Although trehalose is widely distributed in the nature (including prokaryotes and eukaryotes) this sugar has been isolated from a few plant species, being identified in ripening fruits of species from the Apiacea family, in the leaves of *Selaginella lepidophylla* and its relatives (Goddijn & van Dun, 1999) as well as in *Arabidopsis thaliana* (Wingler *et al.*, 2002). According to Elbein *et al.* (2003) in yeast and plants trehalose may serve as a signaling molecule to direct or control certain metabolic pathways or even to affect growth. In addition, it has been shown that trehalose can protect proteins and cellular membranes from denaturation caused by a variety of stress conditions, including desiccation. Kaushik & Bhat (2003) demonstrated that this sugar is an exceptional stabilizer of proteins, while Fait *et al.* (2006) considered its role in the maintenance of the conformation of both storage and housekeeping proteins during dehydration in seeds of *A. thaliana*.

Considering the evidences in favor of a positive role of this protein under abiotic stress, trehalose has been widely evaluated in expression assays. Garg *et al.* (2002) showed that trehalose overproduction has considerable potential for improving abiotic stress tolerance in rice transgenic plants, which accumulated increased amounts of it and showed high levels of tolerance to salt, drought, and low-temperature stresses, as compared with the non-transformed controls. Resurrection plants (*S. lepidophylla*) have the ability to withstand almost complete water loss in their vegetative tissues, being able to remain alive in the dried state for several years and regaining full functionality upon re-hydration (Scott, 2000). Such capacity is associated with an accumulation of trehalose in plant leaves (Iturriaga *et al.*, 2000).

Almeida *et al.* (2005) transformed tobacco plants with the *AtTPS1* gene from *Arabidopsis*. The transgenic seeds were germinated on media with different concentrations of mannitol (0, 0.25, 0.5 and 0.75 M) and sodium chloride (0, 0.07, 0.14, 0.2, 0.27 and 0.34 M) to score their tolerance to osmotic stress. Additionally, the transgenic plants were submitted to drought, desiccation (measurement of water loss as a consequence leaf detaching) and temperature stresses (germination at 15 °C and 35 °C). The transformed plants revealed a reduced increase of drought tolerance and dehydration but exhibited a considerable tolerance to osmotic and temperature stresses, indicating that the heterologous expression of *TPS1* gene from *Arabidopsis* can be successfully used to increase abiotic stress in plants.

Zhang *et al.* (2005) transformed tobacco plants with the trehalose synthase gene from *Grifola frondosa*, submitting transformed plants to drought and salinity stresses (MS medium containing 1 % NaCl). Compared with non-transgenic plants, the transgenic ones were able to accumulate high levels of trehalose, which were increased up to 2.126–2.556 mg/g fresh weight, although levels were undetectable in non-transgenic plants. This trehalose accumulation resulted in increased tolerance to drought and salinity improving physiological performance, such as water content in excised leaves, malondialdehyde content, chlorophyll a and b contents, the activity of superoxide dismutase (SOD) and peroxidase (POD) in excised leaves.

Some evaluations of trehalose activity have been also carried out in sugarcane. Wang *et al.* (2005) transferred the trehalose synthase gene from *G. frondosa* to sugarcane, analyzing the tolerance of the transgenics to osmotic stress [PEG8000 17.4 % (w/v)]. While the non-transformed plants began turning yellow at the third day, with wilting and drying extending from old leaves to young leaves in seven days, all transgenic plants kept green and began turning yellow only at the seventh day, indicating their improvement regarding osmotic stress tolerance. Zhang *et al.* (2006) carried out a similar approach using the same gene in sugarcane, generating transgenic plants that accumulated high levels of trehalose

(up to 8.805–12.863 mg/g fresh weight), whereas trehalose was undetectable in non-transgenic plants. Trehalose accumulation in these plants resulted in increased drought tolerance, as shown by the drought physiological indexes, such as the rate of bound water/free water, plasma membrane permeability, malondialdehyde content, chlorophyll a and b contents, and activity of SOD and POD of the excised leaves.

5. SuperSAGE: Looking for osmoprotectants in sugarcane

Besides a review of the up to date evaluations, the present preview analyzes the sugarcane transcriptome under drought, using a combination of high-throughput transcriptome profiling by SuperSAGE with the Solexa® sequencing technology, allowing the *in silico* identification of potential tags related to osmoprotectants in response to this stress. In the scope of this report four libraries have been generated by the Bulked-Extremes SuperTag Digital Gene Expression (BE-STDGE) method (GenXPro GmbH, Frankfurt, Germany), using bulked root tissues from four drought-tolerant materials as compared with four bulked drought-sensible genotypes, aiming to generate a panel of differentially expressed stress-responsive genes. Both groups were submitted to the same experimental conditions at the glasshouses of CTC (a Brazilian Sugarcane Technology Center, in Piracicaba, state of São Paulo, Brazil), including 24 hours of water deficit stress as compared with non stressed controls. The SuperSAGE libraries produced 8,787,315 tags (26 bp) that, after exclusion of singlets, allowed the identification of 205,975 unitags. Most relevant BlastN matches (42 ≤ Score ≤ 52; intact CATG sequence and plus/plus alignments) comprised 567,420 tags, regarding 75,404 unitags with 164,860 different ESTs, most of them matching to sequences of the genus *Saccharum*. The coverage of the transcriptome by the tags, considering the number of tags per genotype in relation to the number of expected transcripts per cell (500,000; Kamalay & Goldberg, 1980), was 6.5 times for the tolerant and 5.8 for the sensible bulk, i.e., the number of expected transcripts in a single copy per cell should be represented by around six tags in each library. Coverage of this magnitude permits a comprehensive evaluation of a given transcriptome, including rare expressed transcripts.

5.1 Betaine aldehyde dehydrogenase (BADH)

Regarding BADH (EC 1.2.1.8) seven unitags were identified. Most of them (six) were upregulated (UR) in the first comparison (I) that refers to the bulk of tolerant accessions under stress *versus (vs)* non stressed tolerant control (Table 1). From these UR unitags, only one (SD186519) was also UR in the comparison II (sensible bulks under stress *vs* the sensible control; Table 1). This unitag was aligned (BlastN) with accession gb A275267.1 and TC139975 (SOGI, *Sacharum officinarum* Gene Index, release 3). The first EST refers to a cDNA showing a perfect match while the second regarded a transcript similar to a BADH (UniRef100_Q6BD86) with a single mismatch. Two other UR unitags in the tolerant bulk (SD161066 and SD158219) showed contrasting expression (DR) as compared with the susceptible bulk (comparison II; Table 1). The SD158219 unitag aligned with the same TC139975 of SOGI database, in the 3'UTR region regarding the BADH cDNA of maize (gb BT067636.1). Alignments of tags in the 3'UTR region are expected when using this methodology that is based on cDNAs originated from the vicinity of the poli-A tail of RNAs.

Comparison Libraries		I DTS/DTC		II DSS/DSC		III DTS/DSS		IV DTC/DSC	
Tag	Annotation	FC	Reg	FC	Reg	FC	Reg	FC	Reg
SD186519	BADH	2.2	UR	1.6	UR	1.80	UR	1.33	ns
SD161066	BADH	1.3	UR	-1.4	DR	2.81	UR	1.58	UR
SD158219	BADH	6.4	UR	-5.3	DR	6.36	UR	-5.32	DR
SD160278	BADH	2.0	UR	-1.4	ns	3.44	UR	1.27	ns
SD167799	BADH	2.1	UR	-1.7	ns	1.57	ns	-2.20	DR
SD167796	BADH	3.4	UR	1.1	ns	2.04	ns	-1.51	ns
SD7041	BADH	1.2	ns	-1.3	ns	2.69	UR	1.64	ns
SD68048	P5CS	3.3	UR	4.7	UR	2.0	ns	2.8	UR
SD130985	P5CS	5.8	UR	-1.1	ns	1.2	ns	-5.2	ns
SD154736	P5CR	1.3	ns	-1.1	ns	1.3	ns	-1.1	ns
SD175871	P5CR	1.2	ns	-1.7	ns	1.3	ns	-1.6	ns
SD50849	MIPS	1.3	UR	-2.9	DR	2.22	UR	-1.71	DR
SD50847	MIPS	1.1	ns	-2.3	DR	-1.03	ns	-2.50	DR
SD134872	MIPS	1.1	ns	-3.2	DR	#	ns	-3.41	ns
SD61158	TPS	2.4	UR	1.8	ns	3.62	UR	2.80	UR
SD146286	TPS	2.5	ns	2.8	UR	1.28	ns	1.41	ns
SD267553	TPS	-1.3	ns	-2.7	ns	-1.76	ns	-3.73	DR
SD6994	TPP	6.3	UR	-1.8	DR	7.37	UR	-1.50	ns
SD25600	TPP	2.4	UR	#	ns	2.38	UR	#	ns
SD190162	TPP	7.1	UR	1.5	ns	8.50	UR	1.76	ns

Alt Tra: Alternative transcript version; Chr: chromosome; put: putative; exp.: expressed; Align: Alignment Length; Mis: Mismatch; Orient: Orientation. BADH (Betaine aldehyde dehydrogenase); P5CS (Delta(1)-pyrroline-5-carboxylate synthetase); P5CR (Delta(1)-pyrroline-5-carboxylate reductase); MIPS (Myo-inositol 1-phosphate synthase); TPS (Trehalose-6-phosphate synthase); TPP (Trehalose-phosphatase protein)

Table 1. Comparison of sugarcane SuperSAGE libraries showing tags annotated as osmoprotectant-relative, the respective fold change, and regulation of the tags ($p \leq 0.05$).

On the other hand, the SD161066 unitag aligned with two mismatches in the TC24905 of the PAVIGI (*Panicum virgatum* Gene Index, release 1). This TC presented an annotation against a partially homologue (85 %) *Zea mays* sequence of betaine aldehyde dehydrogenase.

All the unitags were aligned against the *Sorghum bicolor* genome available at the Phytozome site (http://www.phytozome.net/) and the respective cDNAs. From seven BADH unitags three mapped on the genome (SD161066 at chromosome 7; SD160278 and SD7041 both at chromosome 6) (see the loci at the Table 2). As mentioned by Ming *et al.* (1998), the levels and patterns of chromosome structural rearrangement in *Saccharum* and *Sorghum* based in their close relationship, high degree of colinearity, and cross-hybridization of DNA probes, all impel use of the small genome of *Sorghum* to guide molecular mapping and positional cloning in *Saccharum*.

For each identified locus a single transcript was identified in *Sorghum* (Table 2). Further, as shown in Table 2, the unitag SD7041 presented a perfect BlastN alignment (score 52) of +/- (plus/minus) type against the transcript Sb06g019200.1, the same cDNA that aligned to another unitag in the +/+ sense (in this last case presenting some mismatches). A detailed analysis of the +/- alignment revealed its positioning in a complementary 3'UTR region in

Table 2. BlastN results of SuperSAGE osmoprotectants-related tags from sugarcane roots under hydric deficit against cDNAs of *Sorghum bicolor* (Phytozome database).

Tag ID	Annot	Locus/Alt Tra	Phytozome Annotation	Chr	Identity (%)	Align bp	Mis	Tag start	Tag end
SD160278	BADH	Sb06g019200.1	aldehyde dehydrogenase, put., exp.	6	96	26	1	1	26
SD161066	BADH	Sb07g020650.1	aldehyde dehydrogenase, put., exp.	7	96	26	1	1	26
SD7041	BADH	Sb06g019200.1	aldehyde dehydrogenase, put., exp.	6	100	26	0	1	26
SD130985	P5CS	Sb09g022290.1	amino acid kinase, put., exp.	9	100	22	0	1	22
SD130985	P5CS	Sb09g022290.2	amino acid kinase, put., exp.	9	100	22	0	1	22
SD68048	P5CS	Sb03g039820.1	amino acid kinase, put., exp.	3	96	26	1	1	26
SD154736	P5CR	Sb03g045690.1	pyrroline-5-carboxylate reductase, put., exp.	3	100	26	0	1	26
SD134872	MIPS	Sb01g044290.1	inositol-3-phosphate synthase, put., exp.	1	100	26	0	1	26
SD134872	MIPS	Sb01g044290.2	inositol-3-phosphate synthase, put., exp.	1	100	26	0	1	26
SD134872	MIPS	Sb01g044290.3	inositol-3-phosphate synthase, put., exp.	1	100	26	0	1	26
SD50847	MIPS	Sb01g044290.1	inositol-3-phosphate synthase, put., exp.	1	100	26	0	1	26
SD50847	MIPS	Sb01g044290.2	inositol-3-phosphate synthase, put., exp.	1	100	26	0	1	26
SD50847	MIPS	Sb01g044290.3	inositol-3-phosphate synthase, put., exp.	1	100	26	0	1	26
SD50849	MIPS	Sb01g044290.1	inositol-3-phosphate synthase, put., exp.	1	96	26	1	1	26
SD50849	MIPS	Sb01g044290.2	inositol-3-phosphate synthase, put., exp.	1	96	26	1	1	26
SD50849	MIPS	Sb01g044290.3	inositol-3-phosphate synthase, put., exp.	1	96	26	1	1	26
SD146286	TPS	Sb04g035560.1	trehalose-6-phosphate synthase, put., exp.	4	96	26	1	1	26
SD61158	TPS	Sb04g035560.1	trehalose-6-phosphate synthase, put., exp.	4	96	25	1	1	25
SD190162	TPP	Sb07g020270.1	trehalose-6-phosphate synthase, put., exp.	7	96	26	1	1	26
SD25600	TPP	Sb07g020270.1	trehalose-6-phosphate synthase, put., exp.	7	96	26	1	1	26

Alt Tra: Alternative transcript version; Chr: chromosome; put: putative; exp.: expressed; Align: Ali Orient: Orientation. BADH (Betaine aldehyde dehydrogenase); P5CS (Delta(1)-pyrroline-5-carboxyl pyrroline-5-carboxylate reductase); MIPS (Myo-inositol 1-phosphate synthase); TPS (Trehalose-6-phos phosphatase protein).

the reverse strand as compared with the genome and the transcript, suggesting a putative NAT (natural antisense transcript). NATs are naturally occurring RNA transcripts that are complementary to other endogenous RNA transcripts. They may regard Cis-natural antisense transcripts (cis-NATs) when transcribed at the same genomic loci of other genes, but from the opposite direction, while trans-NATs are transcribed from different genomic *loci* (Lavorgna *et al.*, 2004). Such antisense transcripts do not compose an uniform group, but present some features in common, with emphasis on the complementarity to the sense genic sequences that may (or not) codify proteins (Faghihi & Wahlestedt, 2009). NATs have been reported in different types of expression assays, including SAGE (Quéré *et al.*, 2004), LongSAGE (Obermeier *et al.*, 2009) and SuperSAGE (Molina *et al.*, 2008).

An annotation against the Phytozome regarding the BADH unitags identified them as aldehyde dehydrogenase, a protein family that includes BADH. Considering their absolute frequency observed (from six to 95 tags per million – tpm - in the tolerant bulk), the sensitivity of the SuperSAGE methodology in detecting rare transcripts could be verified in posterior assays.

For most UR unitags (four out of six) in the tolerant bulk (Table 1), the amount of BADH tags after the drought tolerant stresses (DTS) was significantly higher (comparison III) than that observed for the drought sensible stressed (DSS) bulk. In the absence of stress (comparison IV), both bulks, that are genetically diverse, presented variable expression, depending on the unitag. Still, a single unitag (SD7041) presented no significant expression differences after stress in both comparisons (I and III).

5.2 Delta(1)-pyrroline-5-carboxylate synthetase (P5CS)

With respect to P5CS (EC 2.7.2.11) two unitags were induced in the comparison I (stressed tolerant *vs* tolerant control), while in the comparison II (sensible bulk) a unitag (SD68048) appeared induced (Table 1). For this unitag the observed difference among both bulks under stress was not significant at the studied level ($p \leq 0.05$; comparison III), revealing similar amounts in both bulks 24 hours after drought stress. The same unitag (SD68048) was also UR in the comparison IV (both controls without stress), being significantly most represented than in the tolerant bulk (Table 1).

In turn, the SD130985 unitag was UR in the comparison I (tolerant bulk stressed *vs* control), presenting a higher FC than that estimated for the unitag SD68048 (5.8 *vs* 3.3). Both unitags aligned to the 3'UTR region of the associated ESTs with a single mismatch (data not shown). Considering the alignment against the *Sorghum* genome, both tags mapped, with SD68048 in the chromosome 3 (associated with a transcript) while SD130985 mapped in the chromosome 9 in a region corresponding to two alternative transcripts with different sizes regarding their UTR portions, with no consequences to the CDS and the final protein. The absolute frequencies of these unitags in the sugarcane transcriptome via SuperSAGE varied from three to 19 tpm (tags per million) considering their presence in the tolerant bulk.

5.3 Delta(1)-pyrroline-5-carboxylate reductase (P5CR)

For P5CR (EC 1.5.1.2) two unitags have been observed, but these presented no significant variation in the analyzed SuperSAGE comparisons, being therefore not commented here. After mapping both unitags against the *Sorghum* genome, only the SD154736 tag mapped in the chromosome 3 (Table 2) in a CDS region of an identified transcript.

5.4 Myo-inositol 1-phosphate synthase (MIPS)

Concerning MIPS (EC 5.5.1.4), from three annotated unitags only one (SD50849) was overexpressed (UR) in the comparison I, while all three unitags were repressed (DR) in the comparison II (Table 1). All three unitags mapped in the chromosome 1 of *Sorghum* in the *locus* Sb01g044290, with three predicted alternative transcripts. Two of the tags presented perfect alignments (score 52) with the referred *locus*, one of them (SD134872) aligned in the CDS of the three predicted transcripts, while the other (SD50847) aligned in the region covering the transition from CDS to the first four bases of the 3'UTR. The remaining unitag (SD50849) presented a mismatch with the *Sorghum* genome and also with the respective transcript (Table 2). Interestingly, this unitag represents a possible single base polymorphism (A/G substitution) compared to unitag SD50847. Regarding this polymorphism, sequencing errors are not probable, especially considering the tag frequency. Both unitags were the most frequent in the SuperSAGE libraries, varying from 37 to 59 tpm, while the unitag SD134872 presented less than 3 tpm. This potential SNP and its relation to the differential expression in the tolerant (comparison I) is worth additional efforts for its validation in the future.

5.5 Trehalose-6-phosphate synthase (TPS)

Three unitags have been annotated for TPS (EC 2.4.1.15) one of them UR in the comparison I (SD61158) the second UR in comparison II (SD146286) and the third (SD267553) not varying significantly among the different compared conditions (Table 1). All three unitags mapped against the *Sorghum* genome in the chromosomes 4 and 9 (Table 2). Considering the matching region of chromosome 4, a single *Sorghum* transcript was associated (Sb04g035560.1) similar to a putative uncharacterized protein. Compared to this transcript, the SD146286 unitag presented a substitution (G/A) in a CDS region, while the unitag SD61158 presented two G/A substitutions. From the three unitags, SD61158 was the most expressed, varying from 16 to 39 tpm, while the other two tags were less frequent (< 4 tpm). The alignment against chromosome 9 revealed two mismatches as compared with the unitag SD267553.

5.6 Trehalose-phosphatase protein (TPP)

For trehalose-phosphatase (EC 3.1.3.12) three unitags were UR in the comparison I (Table 1), while one of them (SD6994) was also DR in the comparison II. The mentioned unitag and the SD190162 unitag presented the highest FCs (6.3 and 7.1) for comparison I (Table 1) with the related ESTs sharing 91 % identity in 230 aligned bases. From all three unitags, SD6994 was the most expressed (13 to 83 tpm), followed by SD190162 (3 to 20 tpm) and by SD25600 (< 3 tpm). All three unitags mapped in the sorghum genome, in the chromosome 7, aligning with the transcript Sb07g020270.1 (Table 2) annotated as a putative trehalose-6-phosphate synthase. The most expressed unitag was SD6994 aligned perfectly with a *Sorghum* transcript at the 3'UTR portion of the sequence. Besides, the unitag SD25600 is similar to the SD6994, with a single A/C substitution. The third unitag (SD190162) also aligned in the 3'UTR of the same *sorghum* transcript in a more distant position in comparison to the 3' end than SD6994 presenting a single mismatch to the *Sorghum* transcript (T in *Sorghum* and C in the tag). If the alignment was perfect, one could argument that it was the consequence of an incomplete digestion by the *Nla*III enzyme. However, in the present work a double digestion was carried out, avoiding this error source.

6. Concluding remarks

The present review highlights how scarce information about sugarcane osmoprotectants are at physiological, genomic and transcriptomic levels. Many crops lack the ability to efficiently synthesize some types of osmoprotectants that are naturally accumulated by stress-tolerant plants. Our SuperSAGE data revealed that all procured osmoprotectants categories are present and expressed in sugarcane. However, most of them are discretely expressed in roots after 24 hours of drought stress and also considering the same tissue in non-stressed controls. These discrete expression and their fold changes, detected by SuperSAGE, would probably remain undetected using other transcriptomics approaches, justifying the scarce previous informations about this protein group in sugarcane. Some identified candidates may have an osmoprotectant role in the initial response against drought in this crop and deserve additional evaluations. Hence, as shown by different research approaches in plants lacking osmoprotectants, their transgenic expression represented dramatic differences in the tolerance and survival to abiotic stresses including drought, salinity and freezing, what may be the case of sugarcane. The present chapter brings the first overview of the sugarcane transcriptome under drought with a combination of the high-throughput transcriptome profiling SuperSAGE technology coupled with a next-generation sequencing platform. This approach allowed the identification of some potential target osmoprotectants candidates in the drought stress response. Validation procedures as well as transient expression assays are planned for future works, aiming to collaborate with breeding and biotechnological approaches for benefit of the sugarcane culture, especially facing the scenario of future climate change.

7. Acknowledgments

8. References

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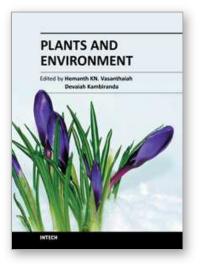
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Plants and Environment

Edited by Dr. Hemanth Vasanthaiah

Changing environmental condition and global population demands understanding the plant responses to hostile environment. Significant progress has been made over the past few decades through amalgamation of molecular breeding with non-conventional breeding. Understanding the cellular and molecular mechanisms to stress tolerance has received considerable scientific scrutiny because of the uniqueness of such processes to plant biology, and also its importance in the campaign â€œFreedom From Hungerâ€. The main intention of this publication is to provide a state-of-the-art and up-to-date knowledge of recent developments in understanding of plant responses to major abiotic stresses, limitations and the current status of crop improvement. A better insight will help in taking a multidisciplinary approach to address the issues affecting plant development and performance under adverse conditions. I trust this book will act as a platform to excel in the field of stress biology.

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