Cadmium accumulation in tomato cultivars and HMA1 characterization in cv. Micro-Tom

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Key words: tomato, cadmium, accumulation, HMA1, VIGS

Cadmium (Cd) is one of the most dangerous trace elements to the environment and human health, taking into consideration that plants grown in contaminated soils are able to absorb and accumulate the metal in their tissues. Plant edible tissues constitute the main pathways for Cd entry into the food chain. Thus, the present study aimed to quantify and compare the Cd content in leaves and fruits of six tomato cultivars following exposure to Cd, as well as to characterize genes possibly involved in Cd tolerance and accumulation in this species. To this end, the tomato cultivars were grown under Cd treatment (90 mg Cd kg⁻¹ substrate) or under the control condition in which Cd was not added. Simultaneously, a search for orthologs genes of Arabidopsis thaliana HMAs (HMA1 - HMA4) was performed. These genes form a group of metal transporters, including Cd. By this search, was identified an ortholog of A. thaliana HMA1 gene in tomato. The six tomato cultivars used were characterized as Cd hyperaccumulators and were able to accumulate Cd in the fruits. In order to characterize the role of HMA1 during the process of Cd hyperaccumulation in tomato cv. Micro-Tom, this gene was used in the steps that comprise the virus induced gene silencing (VIGS). The silencing of HMA1 had no effect on Cd accumulation in tomato shoot. On the other hand, this gene silencing resulted in a reduction in dry weight of the roots and also increased the intensity of chlorosis symptoms in tomato plants grown with 0.7 mM of Cd. Taken together, the results indicate that tomato represents a possible pathway for Cd entry into the food chain and an attractive model organism for the elucidation of the mechanisms involved in Cd hyperaccumulation.

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A DOUBLE KNOCKDOWN OF CHLOROPLASTIC ASCORBATE PEROXIDASE IN RICE TRIGGERS CHANGES IN THE METABOLISM UNDER STRESS BUT PERMITS NORMAL PLANT DEVELOPMENT

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Keywords: Oryza sativa; Gramineaea; Rice; APX; Ascorbate peroxidase; Photosynthesis; Oxidative stress.

The inactivation of the chloroplast ascorbate peroxidases (chlAPXs) has been thought to limit the efficiency of the water-water cycle and photo-oxidative protection under stress conditions. In this study, we have generated double knockdown rice (\textit{Oryza sativa} L.) plants in both \textit{OsAPX7} (sAPX) and \textit{OsAPX8} (tAPX) genes, which encode chloroplastic APXs (chlAPXs). By employing an integrated approach involving gene expression, proteomics, biochemical and physiological analyses of photosynthesis, we have assessed the role of chlAPXs in the regulation of photosystem I (PSI) and photosystem II (PSII) and photosynthesis of rice plants exposed to high light (HL) and methyl viologen (MV). The chlAPX knockdown plants were affected more severely than the non-transformed (NT) plants in photochemical activity and CO\textsubscript{2} assimilation in the presence of MV, suggesting that these enzymes are important for PSI and its associated components. Although MV induced significant increases in pigment content in the knockdown plants, the increases were apparently not sufficient for protection. Treatment with HL also caused generalized damage in PSII in both types of plants. The knockdown and NT plants exhibited differences in photosynthetic parameters in response to light and intercellular CO\textsubscript{2} pressure under normal growth conditions. The knockdown plants overexpressed other antioxidant enzymes in response to the stresses. Our data suggest that a partial deficiency of chlAPX expression modulated the PSII activity and integrity, reflecting the overall photosynthetic process when rice plants are subjected to acute stress at the level of PSI. However, under normal growth conditions, the knockdown plants exhibit normal phenotypes, biochemical and physiological responses.

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FUNCTIONAL CHARACTERIZATION OF THE RICE PEROXISOMAL ASCORBATE PEROXIDASE 4

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Keywords: rice, ascorbate peroxidase, peroxisome, antioxidant system, gene expression.

Reactive Oxygen Species (ROS), like hydrogen peroxide (\(H_2O_2\)), are continually produced by aerobic metabolism. ROS act as signalling molecules and are able to regulate the expression of stress responses, senescence, programmed cell death, plant growth and development, among other metabolic processes. But, in high levels, ROS are cytotoxic. Plant cells developed mechanisms to tightly regulate the intracellular concentration of these molecules. As a central component of the major hydrogen peroxide detoxifying system in plant cells, the ascorbate-glutathione cycle, ascorbate peroxidases (APx) play an essential role in the control of intracellular ROS levels. The present study aimed to characterize the function of peroxisomal APx4 isoform in rice plants. The strategy was to produce rice plants expressing interference RNA for the OsAPx4 gene to describe the silenced plants phenotype and to characterize the gene expression profile in different plant development stages. To produce silenced plants it was used the pANDA vector and the genetic transformation was done via A. tumafaciens. The silencing of rice OsAPx4 gene resulted in plants with a reduced number of seeds and showed a delay in panicle development, when compared to the non-transformed plants. In addition, these plants were more sensitive to senescence, presenting a greater number of senescent leaves, lower chlorophyll content and changes in the expression levels of senescence related genes. Biochemical analyses have shown a modulation of other antioxidant system enzymes. The expression analysis revealed that OsAPx4 gene present higher expression in leaves, but also is expressed in roots and panicles of rice. Analysis of the OsAPx4 promoter revealed expression in leaves, vascular button and panicle, mainly in the conducting vessels. In situ hybridization experiments in panicles showed expression in lemma and peduncle. Based on these results it appears that OsAPx4 gene has its function related to the functions assigned to the peroxisome and probably acts in the physiological processes regulation, such as senescence. APx enzymes are part of the complex plant antioxidant system and these results may contribute to a better understanding of the APx role in the cellular metabolism and plant defense.

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CHANGES IN THE EXPRESSION OF LIGNIN-RELATED GENES IN MAIZE SHOOTS FROM SEEDS PRIMED WITH ASCORBIC ACID

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The modulation of lignin biosynthesis is related to tolerance for several abiotic stresses, however, high lignin content can have a negative aspect due to some properties that present obstacles to chemical pulping, forage digestion, and conversion of lignocellulosic material into biofuels. Therefore, it is of wide interest to generate plant material with lower lignin content and/or with easier accessibility of bio-energetically valuable compounds, in addition to tolerance to various stresses. Previously, we demonstrated that treatment of maize seeds with ascorbic acid in powder induces tolerance for a specific abiotic stress (aluminum in soil). At the same time, this method increased the expression of key genes related to lignin biosynthesis in the roots. In this current study, by quantitative Real-Time-PCR (RT-qPCR), we observed that an opposite response occurs in the shoots since the treatment of seeds with ascorbic acid resulted in decreased \textit{comt1} and \textit{f5h} gene expression, which encode two enzymes of the lignin pathway, and a difference in the expression pattern of transcription factors controlling lignin genes comparing roots and shoots. After treatment with ascorbic acid and under aluminum stress, the negative regulator \textit{ZmMYB31} displayed a reduction of expression in the roots, however no change was observed in the shoots. A second transcription factor, \textit{ZmMYB42}, exhibited lower expression in shoots and no differences in roots under stress. There is evidence that the repressor \textit{ZmMYB31} plays an important role in roots regulating the expression of \textit{comt1} and \textit{f5h}, however in shoots, several other transcription factors could be involved additionally in the control of lignin. In conclusion, the treatment of maize seeds using ascorbic acid appears to be a simple and cost effective technique that can be used to induce aluminum tolerance in maize and, at the same time, reduce expression of genes related to lignin biosynthesis in shoots, thus having the potential to increase yield of cellulose, roughage and biofuels not only in maize. Future field studies are necessary to establish if this treatment affects further aspects of the treated plant. Additionally, it has to be determined if this treatment results in similar responses for other types of stresses, whether abiotic or biotic.
IDENTIFICATION OF GENES ASSOCIATED WITH COLD TOLERANCE IN *INDICA* RICE GENOTYPES DURING THE VEGETATIVE STAGE

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Key words: cold tolerance, gene expression, *indica* rice, *Oryza sativa*, vegetative stage.

The rice cultivars released by research in Brazil are mostly belonging to the *indica* variety. They have high yield potential and grain quality, but are extremely sensitive to cold. In the state of Rio Grande do Sul, the incidence of low temperatures during the early stages of development is one of the major limiting factors to rice productivity. About one hundred *indica* genotypes were evaluated according to the survival percentage of plants at three leaf-stage after ten days at 10°C (in a growth chamber) and seven days of recovery under normal temperature (in greenhouse conditions). The genotypes IRGA 959-1-2-2F-4-1-4-A and IRGA 959-1-2-2F-4-1-4-D-1-CA-1 were characterized, respectively, as tolerant and susceptible to low temperature stress. The objective of this work was to analyze the expression level of cold-related genes (previously described in literature) which could explain the tolerant/susceptible characteristics. Rice plants of the genotypes characterized as susceptible and tolerant to cold were maintained at 10 °C for six hours. Specific primers to fourteen rice genes expected to be involved in cold tolerance were designed to assess gene expression level by qRT-PCR. Tolerant and susceptible genotypes exhibited different levels of *LIP9*, *WCOR413* and *DREB1B* gene expression. Expression of *LIP9* was higher in the tolerant genotype after six hours of cold, whereas *WCOR413* expression was highly inhibited by cold in both genotypes. *DREB1B* expression increased after six hours of cold mainly in the tolerant genotype. Additionally, two other genotypes characterized as susceptible (IRGA416 and IRGA2402-4-1T-9-MF) and tolerant (CT10992-3-4-1T-3P-2P-3 and IR63372-02) were subjected to cold-stress to validate the differential expression previously found. *LIP9* and *DREB1B* genes also showed higher expression in the tolerant genotype CT10992-3-4-1T-3P-2P-3 after six hours of cold, while *WCOR413* expression was lower in the IR63372-02 tolerant cultivar. The results indicate that expression of *LIP9* and *DREB1B* genes is highly induced by low temperature stress in two cold tolerant genotypes. These genes can be used as targets in future investigations underlying the molecular mechanisms of cold tolerance in rice.
Currently the sugarcane is a crop of greater economic importance, since it is a source of feedstock for ethanol production, sugar and biomass. Among the factors that negatively influence the productivity of sugarcane, drought stress is the principal. To better understand the mechanisms of response to drought, was conducted a microarray experiment in field conditions and a RNAseq experiment in greenhouse conditions, using two contrasting sugarcane varieties, sensitive ‘IACSP97-7065’ and tolerant to drought ‘IACSP94-2094’ in different harvest times. The experiments resulted in the identification of numerous transcription factors (TFs) among other transcripts. The microarray and RNAseq experiments resulted respectively in 22 and 118 TFs differentially expressed. Several classes of transcription factors were identified (AP2/EREBP, bZIP, HLH, MYB, NAM and ZFP) and among them, five (bZIP, HLA, MYB, NAM and ZFP) were selected for qPCR validation. In field conditions, bZIP, HLA and ZFP showed the highest expression level (up-regulated) after 90 days of drought for both sugarcane varieties compared with 30 and 120 days of drought. Only NAM showed the same level expression (up-regulated) in materials harvested at 90 and 120 days for both sugarcane varieties. MYB expression was down regulated in both sugarcane varieties with the lowest expression level after 120 days of drought. In greenhouse conditions, HLA, MYB and ZFP were down-regulated in both sugarcane varieties showing the lowest level of expression at 90 days of drought. bZIP was up-regulated reaching the highest level of expression after 90 days of drought for both sugarcane varieties. This study identified transcription factors responsive to drought, allowing the choice of TFs with high potential to be used for transformation in models plants as rice (Oryza sativa).

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Biofuels has receiving an important attention as it is a renewable source, and because of this castor bean (*Ricinus communis* L.) became an important plant for oil production with high potential for biodiesel. Furthermore, castor bean has been suggested to grow at the Brazil northeastern, where water availability may be reduced. Considering this, the aim of this work was to identify messengers differently expressed in seeds from plant submitted to drought stress using subtractive cDNA libraries. In order to do this, castor bean plants (BRS Energy cultivar) were grown at UFRN and drought treatment was done when plants were producing fruits (approximately 120 days old). Drought stress was conducted with 5 and 10 days. Seeds were collected and it were frozen in liquid nitrogen and kept at -80°C freezer for molecular analyses. It was done three cDNA subtractive library according to Super SMART PCR cDNA Synthesis Kit and PCR-Select cDNA Subtraction Kit (Clontech). The results showed that inserts were ranging from 300-650 bp. A total of 592 sequences were obtained from these three libraries. These sequences were analyzed using blastx and GO system. It was identified for 5 days, 10 days and control (no stress) libraries the following metabolic classes respectively: Translation (29-9-14), Seed maturation and nutrients storage (13-18-2), stress response (109-16-96), Energy production (30-16-27), fatty acids synthesis (5-2-1), metabolic pathways (21-10-10), transcriptional process (13-7-8), signal transduction cascade (5-0-0), defense response (38-11-20), metallic ions (2-2-0), Mitochondrial Proteins (5-12-1), Chloroplast proteins (2-2-0), Transposable elements (2-1-3), unknown proteins (8-9-12). Two proteins from stress response class were interesting: Dehydrin Xero, that is involved in drought tolerance and it was also identified five different types of Abundant Late Embryogenesis proteins (LEA), that were closely associated to seed protection against the effects of drought, high salinity or cold. Moreover, it was also identified proteins as SOD, SUMO, heat shock, DNAJ (a chaperone protein) and a cold acclimation protein (CAP160), which modify the cell surface in order to keep membrane and cytoskeleton integrity and reduce the negative effects from drought stress. Other protein identified was Puroindoline b (63 sequences – 35, 8, 20). This protein has been associated to seed storage. Besides the sequence analysis a anatomical and biochemistry enzymatic assays will be done in order to understand the drought effect on seed development and on plant physiology.

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DIFFERENTIAL LEAF PROTEOMIC ANALYSIS UNDER EFFECT OF DROUGHT ON SOYBEAN SEED PROTEIN AND OIL CONTENT

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Palavras-chave: drought, soybean, proteomics, oil content, protein content, seed.

Understanding the mechanisms how plants respond to water stress during reproductive development period is crucial for predicting the impacts of climate change on seed quality. The plants have signaling mechanisms orchestrating responses to differential accumulation of oil and protein content in the seeds. At the R5 stage, variety supreme was submitted to the different regimes of water deficit gradually to 40% of available water in soil. The predawn leaf water potential ($\Psi_{am}$) was measured daily until the potential of -1.5 MPa, where physiological parameters were analyzed and collected the leaves, followed by re-irrigation, where seeds were collected in R8 stage. The oil content and protein content in soybean seeds were analyzed by near infrared spectrometer (FT-NIR equipment Thermo Scientific Antaris II model). The proteomic analysis by two-dimensional electrophoresis was followed by sequencing mass spectrometry (MALDI-TOF-TOF), peptides were identified using the Peptide Mass Fingerprinting Algorithm (PMF) and MS / MS Ion Score, contained in the program Mascot 4®. Data mining was done in NCBI non-redundant (Viridiplantae) protein database. The differential proteomic analysis of leaves under water deficit conditions showed differential expression of proteins involved in metabolism following: carbohydrates, hormones, energy, lipid, glutamate, aspartate, alanine, nitrogen, glutathione and glyoxylate; programmed cell death; responses to abiotic stresses; on the cellular detoxification, oxidative stress and processing protein (post-translation modifications). With the results of proteomics, some proteins selected using the real time quantitative PCR (RT-qPCR) were analyzed. Glutamine synthetase, transketolase, ferredoxin NADP reductase, L-ascorbate peroxidase, oxygen-evolving enhancer protein-2, carbonic anhydrase, phosphoglycerate kinase, aldo-keto reductase 4, quinone oxidoreductase, glyceraldehyde-3-phosphate dehydrogenase, chlorophyll a-b binding protein 3, metalloendoproteinase 1, cytochrome b6-f complex iron-sulfur subunit; LEA-2 (desiccation-related protein) were down-regulated. Abscisic stress ripening gene was up-regulated. This study provides a dynamic view of the effect of drought on soybean leaves and on accumulation of oil and protein content in seeds.

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MOLECULAR REGULATION OF AMMONIUM TRANSPORT SYSTEM BY CARBON STATUS IN SUGARCANE (SACCHARUM SPP.) PLANTS

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Keywords: nitrogen, uptake, gene expression

Nitrogen (N) uptake by plant roots is a highly regulated process and is coordinated with photosynthesis during plant growth and biomass production. During the light period, plants use carbohydrates that are delivered by photosynthesis for growth. In the dark, growth depends on resources that have been stored in the preceding light period. Diurnal changes of sugars and starch provided by photosynthetic activity exert a pivotal role controlling N metabolism. Therefore, a comprehensive understanding of the molecular basis of carbon (C) and N interaction is crucial for improvement of crop yield. Considering that ammonium is a preferential soil N source for field grown sugarcane (Saccharum spp.) plants, herein we describe the diurnal and/or C regulation of the ammonium transport in this specie. Short-term influx of 15N-labeled ammonium into sugarcane roots showed low uptake rates at the beginning of illumination. However, towards the end of the light period ammonium influx increased 20% which was followed by a sharp decrease of 50% at the beginning of dark. An addition supply of 3% sucrose restores the root 15N-ammonium influx during the dusk. These results suggest a diurnal and C regulation of ammonium uptake in roots. The identification of six sugarcane orthologous membrane proteins AMMONIUM TRANSPORTERS/METHYLAMMONIUM PERMEASE (AMT/MEP) prompts us to characterize the regulation of these high affinity ammonium transporters according to diurnal cycle. The increased expression of ScAMT1;1 and ScAMT1;3 correlates with ammonium uptake during the light period. After dusk, both AMTs showed a down-regulation at transcriptional level. Therefore, the diurnal regulation of ammonium uptake in sugarcane roots at the end of the photoperiod seems to be caused by transcriptional up-regulation of ScAMT1;1 and ScAMT1;3. Further, sucrose supply during the dark prevents the down-regulation of both ammonium transporters, which indicates C-dependent regulation of AMTs in sugarcane. Differential regulatory features were observed for the AMTs genes in source leaves. Whilst ScAMT2;3 displayed increased transcript levels during the light period, ScAMT1;3, ScAMT2;1, ScAMT3;1 and ScAMT3;2 showed its maximum expression levels at dark. Among these, ScAMT1;3 showed an unique regulation by external C-supply. Overall, these results indicate a differential regulation of ammonium transporters according to diurnal cycle and C status in sugarcane leaves. Thus, a coordinated regulation of ammonium transport in roots and shoots ensures inorganic N to be diverted to C skeletons that are needed to N assimilation into amino acids synthesis and other N compounds during plant growth.

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CHARACTERIZATION OF CADMIUM TOLERANCE IN TOMATO PLANTS

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Keywords: heavy metal, stress tolerance, reactive oxygen species, Solanum lycopersicum L., tolerance index

Environmental contamination is currently a global problem. Soil contamination by heavy metals has increased in more recent years associated to demands of technologies that require these contaminant elements. Metals, such as cadmium (Cd), are present in batteries, fertilizers, sludge, residues of factories, and others. This metal is normally found in low concentrations in the environment and is toxic to microorganisms, plants and animals. Several studies have focused on the responses of crops under heavy metals stress. This research evaluated the responses of two tomato cultivars (cv. Calabash Rouge - CR and cv. Pusa Ruby - PR) under Cd stress, first by growth rate and metal accumulation in plant tissues, and then by stress level indirectly estimated through MDA and H₂O₂ quantification. Twenty five days old tomato seedlings were exposed to 0 and 50 µM CdCl₂ for four days in Hoagland and Arnon nutrient solution (25% of strength). The results indicated that Cd interfered in tomato plant development causing growth reduction of both tomato cultivars, considering that PR is less affected than CR. This metal accumulated in all tissues in both cultivars, but Cd accumulation was higher in shoots of PR, while in roots the concentration was higher in CR. This result suggests that roots and shoots of PR were possibly less damaged by Cd presence, allowing a higher translocation and tissue accumulation of Cd in this cultivar. Hydrogen peroxide content increased significantly in leaves and root of both cultivars while MDA content increased significantly only in the leaves of both cultivars. Therefore, the tolerance index (TI), which considers the differences of potential growth per se of each cultivar, allowed us to classify PR and CR as Cd-tolerant and Cd-sensitive cultivars, respectively. Finally, ongoing studies are being performed in order to elucidate the Cd-tolerance mechanisms in tomato plants.

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ASR5 AND ALUMINUM TOLERANCE IN RICE: ITS BINDING ACTIVITY IN THE PROMOTER OF STAR1 AND OTHER ALUMINUM RESPONSIVE GENES

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Keywords: Aluminum, ChIP-Seq, RNA-Seq, Rice, ASR

Aluminum (Al) toxicity in plants is one of the primary constraints in crop production. Al³⁺, the most toxic form of Al, is released into soil under acidic conditions and causes extensive plant damage, especially in the roots. Recent studies have identified several Al-responsive genes, including the rice gene ASR5, which plays an important role in rice Al tolerance. Here, we used high-throughput analyses and showed a large-scale profile of Al-responsive genes in rice. Using ASR5_RNAi plants, a global transcriptome analysis was performed to compare non-transformed and ASR-silenced plants. The results identify several genes affected by ASR5 silencing. Chromatin immunoprecipitation followed by deep sequencing revealed the binding motif for ASR5 and demonstrated that ASR5 can act as transcription factor in rice to regulate gene expression during Al stress, including STAR1.

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MELEA3 PROTEIN OF CASSAVA (MANIHOT ESCULENTA CRANTZ): EXPRESSION AND ASSAYS OF TOLERANCE TO SALT STRESS IN BACTERIAL SYSTEM

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Keywords: abiotic stress, cassava, LEA protein, salt stress tolerance, heterologous expression bacteria

Low temperature, drought and high salinity are common stress conditions that adversely affect plant growth and crop production. Late embryogenesis abundant (LEA) proteins are known for their roles in acquisition of tolerance to such abiotic stresses. Previous studies have identified the MeLEA3 cDNA sequence with an increased expression during salt stress in cassava (M. esculenta). Therewith, our aim was to produce the MeLEA3 protein in bacterial system and verify its ability to confer tolerance to salt stress in bacterial cells. Primers containing sites for NdeI and XhoI restriction enzymes were used to clone the MeLEA3 ORF (Open Reading Frame) into the pET29a expression vector generating the pET29a-MeLEA3 construct, which was introduced into Escherichia coli strain Rosetta. Bacterial cells containing the pET29a-MeTCTP construct and an empty pET29a vector (negative control) were cultured in presence of IPTG 1 mM and different NaCl concentrations (0, 250, 500 and 750 mM). Cells growth was measured by evaluation of optical density at 600 nm. SDS-PAGE of total protein of bacteria confirmed an additional protein band with about 10 kDa in the pTE29a-MeLEA3 sample that was not found in the negative control. Our results also revealed that bacterial cells expressing the recombinant MeLEA3 showed a growth advantage during salt treatment in comparison to the control cells. Since salinity is one the most important abiotic factors affecting the production of crops worldwide, the MeLEA3 gene could be a candidate gene for generation of salt tolerant crops.

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Plants are sessile organisms that have to cope with challenges imposed by environmental changes. Although local responses to abiotic stresses have been extensively studied, little is known about the changes in gene expression triggered by a distant stimulus. Here, we investigated the proteome responses of *Eucalyptus globulus* roots against a 24 hours cold stimulus applied only in the shoot and compared the results with a local stimulus treatment. For that, six months old seedlings of *E. globulus* were transferred to an apparatus located inside growth chambers and designed in a way that the root temperature could be independently controlled by a circulating water-bath. Distant proteome responses were induced through temperature adjustment at 10°C for the shoot tissues (growth chamber temperature) and 25°C for roots (treatment 1); while local changes were determined by adjustment at 10°C for shoot and roots (treatment 2). Both treatments were then compared to a control with roots and shoot temperature adjusted at 25°C. Proteins were extracted by phenol partitioning and then separated by two-dimensional electrophoresis. Spots that presented significant densitometric change were selected for analyses in tandem mass spectrometry followed by database-driven protein identification. Image analysis indicated that 14 spots were differentially regulated across the treatments. According to their expression profile they were clustered into 4 groups. Using a concatenated database containing only *E. globulus* and *E. grandis* sequences, we were able to identified proteins from all analyzed spots with high confidence level (1% FDR). As all analyzed spots contained multiple protein identifications, we used a label-free proteomics index (NSAF) to normalize and determine the presence of a dominant protein (s). From the 14 differentially regulated spots, we detected the dominance of 19 proteins which were further reduced to a 16 non-redundant protein list. The group of proteins down-regulated in both treatments was formed by two different heat shock protein isoforms, a chaperonin, a Ran-binding protein and an unknown protein. Different heat shock protein isoforms and a chaperone were down-regulated in treatment 2; while other HSPs, an universal stress proteins, two predicted proteins and an UDP-sugar pyrophosphorylase were up-regulated in the same treatment. Finally, the proteins down-regulated in treatment 1 comprised the following identifications: an actin isoform and a subtilisin-like protease. Independently of the specific function that each of the identified proteins may carry out in the studied system, we can conclude that: the root proteome responds quickly to changes in both local and distant responses (1); although existent, distant tissues are less responsive than locally stimulated tissues (2); root proteome changes to cold stimulus are mainly characterized by down-regulation (3); protein up-regulation responses are specific to local stimulus (4).

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Genomic and transcriptomic studies have contributed to improve the understanding of the molecular mechanisms involved in perception, response and adaptation of the common bean to drought stress. To evaluate global changes in proteins expressed during a drought event and to better understand genotype specific responses, a quantitative shot gun proteomics approach was carried out to three genotypes of common bean (*Phaseolus vulgaris* L.): BAT 477 (tolerant to drought), Carioca 80SH (moderately susceptible) and RAB 96 (susceptible to drought). For this, plants were cultivated under normal conditions until V3 stage and then the water supply was withheld until soil moisture reach 5%. For control plants, water supply was kept during the experiment. Roots were collected from stressed and control treatments and total protein of these samples was obtained by a phenol-based extraction. Label-free quantitative proteomic analysis was carried out in an Ion Trap mass spectrometer, in three biological replicates. After mass spectrometry and protein mapping, an average of 1239 different proteins was identified in each genotype. The comparison between stressed and control samples showed that 36.3%, 33.6% and 50.4% of the detected proteins in RAB 96, Carioca 80SH and BAT 477, respectively, were differentially expressed. A completed annotation for plants was run in Blast2GO software, allowing a very comprehensive analysis of protein classification into Biological Process, Cellular Component and Molecular Function, besides a Singular Enrichment Analysis, that indicated classes of proteins enriched in each genotype under drought. RAB 96 did not display any differential enrichment in GO terms for Biological Process and Molecular Function categories, being the ‘organelle’, ‘intracellular organelle’ and ‘extracellular region’ the unique classes from Cellular Component enriched in this proteome. For Carioca 80SH, four classes were enriched (‘response to stimulus’, ‘response to stress’, ‘metabolic process’ and ‘catalytic activity’) and nine were depleted (‘cellular’ and ‘macromolecule biosynthesis’, ‘gene expression’, translation’, ‘ribonucleoprotein complex’, ‘ribosome’, ‘macromolecular complex’, ‘structural molecule activity’). BAT 477 presented the most active functional regulation considering protein categories, having 24 GO terms enriched and only one depleted, including four classes related to ‘response to stimulus’ and four related to ‘primary’ or ‘secondary metabolism’. Comparing RAB 96, Carioca 80SH and BAT 477 root proteomes under drought, there is a gradual enrichment in stimulus/stress response GO terms according to the tolerance level of the genotype, indicating that the most tolerant genotype can cope with a drought stress using a proportionally richer arsenal of proteins. In addition, up- and down-regulated proteins identified in each genotype were distributed according to the pathways they are related with using KEGG database and MapMan 3.5.1R2 software, allowing a network view of genotype-specific changes in metabolism of common beans under drought stress.
THE USE OF MICRONRNAS AS HOUSEKEEPING GENES IN QUANTITATIVE PCR IN COTTON

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Key-words: microRNA, cotton, housekeeping genes, RT-qPCR

Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) is a technique largely used to investigate gene expression. However, for correct analysis and interpretation of the results, the choice of a suitable gene to normalize the data is a crucial factor. The genes used to normalize expression data, called as reference or housekeeping genes, most have constant expression levels in different tissues and across different conditions. However find genes stable in all situations and that work at the same form in different plant species and conditions is not an easily accessible goal. miRNAs have important regulatory roles in eukaryotic acting in at post-transcription level at different biological functions. In the present study, we evaluated, for the first time, cotton micro RNAs as candidates as reference genes for cotton gene expression studies. The stability of miRNAs in Gossypium hirsutum in different tissues (root, stem, leaf and flower), different commercial cultivars (FiberMax FM966, Delta Opal and Cedro) and under biotic stress caused by infection of Cotton leaf roll dwarf virus (CLRDV) was analyzed. mRNAs already described as reference genes in cotton were also included in these analyzes. Cotton samples were organized in 12 sets where the expression stabilities of the six miRNAs and five mRNA reference genes were evaluated. Four algorithms, geNorm, NormFinder, BestKeeper and DeltaCt were used to identify the stability of these genes and therefore provide an accurate selection of reference genes. In 8 of the 12 sets tested, the micro RNA (miRNAs) were the best housekeeping genes. The best reference genes were validated by expression assays performed in study cases. For that, we studied the expression of miRNAs and mRNA already known to show distinct expression levels in the analyzed experimental conditions. Study cases used leaves of the cotton cultivars FM966 and Delta Opal under stress biotic (CLRDV infection) and flower x leaves from FM966 and Cedro. miR2910, miR164, miR2118, miR2111, miR3476, miR159, GhDCL1, GhDCL2, GhDCL3 and GhDCL4 were evaluated under biotic stress and miR164, GhPGFS7 and GhXTH expressions levels were evaluated in leaves and flowers. Ours results show that the reference microRNA/mRNA genes previously selected work as excellent reference genes in all of the study cases. Statistical test p sustained the expression levels of the differentially expressed miRNA and mRNA even under biotic stress as in flower x leaves. These analysis also showed that to normalize target mRNA the origin, miRNA or mRNA, of the reference gene is not important, but to normalize miRNA expression levels, miRNA reference genes seems to be better than mRNAs. This, use of miRNA for miRNA expressions analyzes instead of mRNAs, was already well supported in human miRNAs studies, however this is the first study were this is shown in plants.

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STUDY OF THE PROMOTER REGION OF THE RICE

**OSAPX1 GENE**

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Keywords: ascorbate peroxidase, promoter expression pattern.

Ascorbate peroxidase (APx) is a key enzyme of the antioxidant metabolism, catalyzing the decomposition of hydrogen peroxide (H₂O₂) in water, using ascorbate as an electron donor. The H₂O₂ is a reactive oxygen species (ROS) produced constantly by aerobic metabolism. Under biotic and abiotic stress the level of H₂O₂ increases and, in large quantities, can cause cellular damage. In rice, there are eight APx genes that encode isoforms target to different subcellular compartments: cytosol, peroxisoma, mitochondria and chloroplast. OsAPx1 gene encodes a cytosolic isoform of APx. The aim of this study was to investigate the expression pattern of the OsAPx1 gene promoter in rice plants. A fragment of 2kb nucleotides preceding the translational initiation site of the OsAPx1 gene was isolated, cloned into pHGWFS7 vector for promoter study. The transformation of rice calli was performed via *Agrobacterium tumefaciens*. Six lines of transgenic plants expressing *gus* under the control of the OsAPx1 promoter were obtained. Samples of the transgenic plants were collected and analyzed by histochemical tests using X-Gluc as substrate. The *Gus* gene expression was observed in leaf mesophyll, ligule and especially in wounded regions. These results show that OsAPx1 gene is expressed in green tissues and respond to damage.

Financial support: CNPq, FAPERGS, CAPES and ICGEB.
CASSAVA TCTP PROTEIN: EXPRESSION AND ASSAYS OF SALT STRESS TOLERANCE IN *ESCHERICHIA COLI*

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**Keywords:** *Manihot esculenta*, abiotic stress tolerance, crop resistance, TCTP expression, bacterial system

Cassava (*Manihot esculenta* Crantz), one of the most important tropical food crops, presents easy propagation system, high drought tolerance, and low demand for nutrients, producing reasonably well under critical climate and soil conditions. There are few studies reporting the expression pattern of genes of this crop, mainly those involved in plant response to abiotic stresses, such as drought and salinity. One of these genes is the translationally controlled tumor protein (TCTP), a multifunctional protein highly conserved and widely expressed in several eukaryotic organisms. Previous studies have identified the MeTCTP cDNA sequence with an increased expression during salt stress in cassava. Therewith, our aim was to express the cassava TCTP gene in *Escherichia coli* in order to evaluate its potential role in response to salt stress. For this, a pair of primers containing sites for *Nde*I and *Xho*I restriction enzymes was used to clone the MeTCTP ORF into the pET29a expression vector, generating the pET29a-MeTCTP construct, which was introduced into *E. coli* strain Rosetta by electroporation. Bacteria cells containing this construct and an empty pET29a vector were induced by IPTG 1 mM with incubation at 37°C for 6 hours, and then used in extraction of total proteins, which were analyzed by SDS-PAGE. For assays of tolerance to salt stress, bacterial cells containing the pET29a-MeTCTP construct and an empty pET29a vector were induced by IPTG 1 mM and submitted to different NaCl concentrations (250, 500 and 750 mM), and cell growth was measured by optical density at 600 nm for 12 h. Our results showed an additional protein band with about 20 kDa in the pET29a-MeTCTP sample that was not found in control sample. Although MeTCTP has a predicted molecular weight of 19 kDa, the additional protein only visualized in the pET29a-MeTCTP sample has a size very close to the 21 kDa predicted molecular weight for the recombinant MeTCTP harboring the His-tag. Also, our results revealed that bacteria over-expressing the recombinant MeTCTP showed a growth advantage during salt treatment in comparison to the control cells in all measured concentrations of NaCl. In conclusion, based on these results, we can infer that MeTCTP expression can play an important role in salt tolerance improvement of cassava.

Financial support: FAPESPA, CNPq, UFPA.
Nitrogen (N) nutrition can profoundly affect plant growth and productivity. In response to limited nutrient availability, plants evolved physiological and developmental plasticity that relies on coordinated pathways to adapt to environmental constraints. These adaptive responses include improving nutrient uptake and recycling, which may alter biomass allocation. Although nitrate is a major N source in agricultural soils, sugarcane has low capacity to use nitrate. Therefore, understanding nitrate uptake and allocation are key factors for improving N use efficiency in sugarcane crops. Here, we report the physiological and the molecular characterization of nitrate transport during N allocation in sugarcane. To assess N allocation in sugarcane under N-limited growth conditions, 3-month-old plants grown in N-replete nutrient solution were submitted to long-term (1d) $^{15}$N-nitrate solution. Next, the sugarcane plants were subjected to N-deficient or N-sufficient (5 mM ammonium nitrate) growth conditions without $^{15}$N which allowed the determination of $^{15}$N fluxes1 and 5d after treatment. In 1d post-labeled plants, most of the previously taken up $^{15}$N was found in roots and stalks and the remaining $^{15}$N were in young leaves. Whilst no significant differences between treatments were observed in $^{15}$N concentrations in roots, N-deficient plants displayed 10% higher $^{15}$N in young leaves than N-sufficient leaves. Long-term N deficiency treatment (5d) showed no translocation of $^{15}$N from roots while N-sufficient plants presented 19% lower $^{15}$N levels. A significant proportion of nitrate-derived $^{15}$N was allocated to young leaves. These results indicate that during N-limited growth conditions root to shoot N transport is impaired in sugarcane. The primary route of long distance nitrate transport relies on a NITRATE/PEPTIDE TRANSPORTER gene family. Based on orthologs from other plant species, we identified the major NRT1 member involved in xylem nitrate loading in the sugarcane EST genome. The xylem loader transporter ScNRT1;5 showed repression in sugarcane roots during N deficiency which correlated with reduced nitrate transport from root to shoot. In addition, the increased expression level for ScNRT1;5 in mature and young leaves supports a lower remobilization of nitrate within leaves. Thus, the regulatory mechanism involving nitrate transport presented here reveals this physiological and molecular feature of nitrate allocation during N deficiency in sugarcane plants.
DIFFERENTIAL TOLERANCE TO ALUMINUM IN SOYBEAN: A STUDY ON THE POSSIBLE INVOLVEMENT OF AQUAPORINS.

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Palavras-chave: aquaporins, aluminum, soil acidity, soybean, differential tolerance

The acidity in the soil, mainly related to levels of aluminum (Al), affects the yield in agricultural production, with the main effect being the reduction of plant root growth. Aluminum tolerance varies between different plant cultivars, which can be classified as tolerant, intermediate and sensitive. The molecular mechanisms involved in the differential tolerance to Al are not yet fully understood. Data obtained with rye indicate a possible relationship between the expression of aquaporins in plant roots and tolerance to Al toxicity, but there aren’t studies that investigate this relationship in more detail. Aquaporins are membrane proteins involved in the transport of water and small solutes through the membrane. In plants, aquaporins are involved, in addition to the transport water, in nutrient absorption, and fixation of carbon and nitrogen. Aquaporins are highly expressed during the cell elongation process, allowing the rapid influx of water into the vacuole, generating the turgor pressure which directs the cell elongation. Analysis of the soybean (Glycine max) genome indicates the presence of at least 58 different aquaporins. This project aims to identify aquaporins that are present in the roots of different soybean cultivars with distinct tolerance to Al, and establish a relationship between the expression levels of genes encoding aquaporins and the difference in the tolerance to Al toxicity. Seeds from the tolerant soybean cultivars Conquista and Williams were germinated on filter paper and, after 3-4 days, the seedlings were transferred to tubes containing nutrient solution. After 96 h in the solution, plants were treated with Al 3 µM and maintained for another 24 h. After the Al-treatment, plants were collected and RNA was isolated from the roots, followed by cDNA synthesis. The same procedure was performed with plants that were maintained in the nutrient solution without Al. The technique of RT-PCR was used to identify the aquaporins present in the roots of the different cultivars. In this first approach, primers for 25 aquaporins were designed and used for the RT-PCR. Aquaporin genes TIP3;3, TIP2;4 and TIP2;5, that showed a distinct expression profile compared to the controls, were further analyzed by real time PCR. From those three aquaporins, TIP2;4 showed the most relevant difference after the Al-treatment, having its expression increased about 5 times in the Conquista cultivar and 3 times in the Williams cultivar, when compared to the untreated plants. Experiments using Al-sensitive plants are underway. Completing this project, we will possibly establish a relationship between the levels of expression of aquaporins in different cultivars and their tolerance to Al. The results obtained in this study may serve as a basis for the development of new approaches to improve plant cultivars, especially soybean, a plant with high economic importance worldwide.

Órgão financiador: FAPERGS, CAPES e CNPq.
Drought, high soil salinity, heating and cold are some of the major abiotic stresses that affect common bean (*Phaseolus vulgaris* L.) productivity in Brazil and other countries. Since its grains are widely consumed due to their nutritional value, it is extremely important to define breeding strategies for abiotic stresses tolerance. Knowledge of the gene pathways involved is crucial to find candidate genes for tolerance improvement. DREB (Dehydration responsive element-binding) transcription factors contain an AP2 domain and are key factors capable to bind and regulate expression of several stress responsive genes. A genome-wide *in silico* search for AP2 domain-containing sequences was performed using data deposited in Phytozome, showing 181 sequences. BLASTn and BLASTx tools were used for proper annotation and just *DREB*-like genes were kept. Sequences were aligned and phylogenetic trees allowed their discrimination into six sub-groups (A-1 to A-6). Gene expression profiles of some of these genes (*PvDREB1*, *PvDREB2A*, *PvDREB5* and *PvDREB6B*) were evaluated under dehydration (PEG 10%), salt (200 mM NaCl), cold (4°C) and abscisic acid (ABA 100 µM) treatments in different times in a drought tolerant genotype, BAT 477. Also, a spatial scale expression analysis was performed comparing BAT 477 and other genotypes: the Andean Jalo EEP558 (drought susceptible); and Mesoamericans BAT 93 (tolerant), IAC-Carioca 80SH (moderately tolerant) and RAB 96 (susceptible). Our qPCR results showed *PvDREB1* was strongly activated (up to 8,000 fold-change) in all treatments in roots, stems and leaves. Low basal transcript levels were found to this gene in untreated plants, but with strong activation immediately after stress induction. *PvDREB2A* was induced by drought in stems (8 fold) and *PvDREB6B* showed activation by drought in roots (2 fold) and leaves (up to 3.4 fold). *PvDREB5* was also induced in all treatments (up to 158 fold). *PvDREB1* and *PvDREB5* were activated by ABA treatment and *PvDREB2A* and *PvDREB6B* were repressed or did not respond to ABA. Expression levels were different among genotypes and interestingly *PvDREB6B* was not expressed and not even amplified in DNA sample of JALO EEP558, suggesting modifications of this gene at the DNA level in this genotype. Those genes seem to be promising for further procedures aiming at breeding strategies for improvement of abiotic stress tolerance in common bean.

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RE-WIRING OF TOBACCO TRANSCRIPTOME BY INTERFERING WITH TNT1 RETROTRANSPOSON EXPRESSION.

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Key words: Tnt1, retrotransposon, gene regulation, transcriptome, tobacco

Retrotransposons are abundant components of plant genomes, able to generate structural and functional variability. These elements can affect gene regulation and genome structure through transposition, recombination of non-homologous regions, and by being targets of epigenetic regulation mechanisms. Their mutagenic character led genomes to develop mechanisms to control their activity. Among these mechanisms the main is methylation either direct or indirect target from RNAi pathway. They can alter expression levels of neighbor genes through the action of their promoters, and generate transcripts alternatively processed through their insertion into the genes. These elements can also modify the methylation state of other genes, transmitting epigenetic marks, and generate siRNAs that will regulate gene expression through transcriptional and post-transcriptional mechanisms. On a global scale, retrotransposons can spread regulatory motifs through the genome, leading to the formation of new gene regulation networks. Due to these features retrotransposons can be considered important players in genomes evolution. While deleterious effects are negatively selected, advantageous changes could be incorporated as mechanisms for gene regulation. Despite the undeniable influence of retrotransposons on their host genomes, only punctual evidences have been shown to suggest a putative role for them in gene regulation to date. In order to investigate this question we compared transcriptome profiles of wild type plants and transgenic lines carrying a hairpin construct targeting the reverse transcriptase of Tnt1 (HP lines). Comparing HP plants with wild-type, 789 genes were modulated among the unigene tobacco dataset. Among these, genes coding for proteins participant in lipid and protein degradation, photosynthetic apparatus, ethylene synthesis and signaling, stress/pathogen response, SAR, chromatin modification, chaperone, as well as transcription factors and signal transduction genes. Furthermore, a subset composed of fifty five genes representing the main biological processes were chosen as targets to infer gene expression networks based on the RNA-seq results. Seven networks not identified in WT plants were observed in HP, proposing the emergence of new expression networks. This result indicates a gene regulation re-wiring in HP plants and suggest a role of Tnt1 in these changes. Our results point out a Tnt1-mediated regulation of several sets of genes, reflecting a wide gene reprogramming. This work introduces a new perspective in the study of transposable elements, aggregating evidences of the mobilome influence on the global transcriptome regulation, and deepening the comprehension of the process through which retrotransposons impact their host genomes.

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CONSTRUCTION OF EXPRESSION CASSETTES FOR OBTAINING RECOMBINANT PROTEINS THAT CONFER RESISTANCE TO SALT STRESS

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Keywords: Metagenome, salt stress, functional Selection, Expression cassette, Recombinant protein.

The microbial diversity of soil known is summarized at 5,000 species, which represents only a negligible fraction of the actual biodiversity. This environment presents a huge untapped biological pool that may be used for discovery of new genes, new metabolic pathways and their products. As approximately 99% of microorganisms may not be cultured by traditional techniques, culture-independent methods are essential to understanding the genetic diversity, population structure and ecological laws of most microbial populations. To resolve this impasse, a new methodology called “metagenomics” has been used. Apart from the soil, the rivers are another ecosystem with high biodiversity, but few were examined using metagenomic libraries. The diversity of bacteria in rivers can be especially high, because they may contain mixtures of terrestrial and aquatic bacteria, including several taxa typical of soil. Some rivers have extreme variations in salt concentration diluted in the water throughout the year causing enormous losses in agricultural production areas near of them. Thus, in previous work a metagenomic library was constructed and a functional screening for new genes that confer resistance to salt was realized. So two clones were selected and after sequenced, the potential ORFs were identified: in one clone were found two ORFs, one related to glucose-6-phosphate isomerase and another related to putative myo-inositol 2-dehydrogenase, in the second clone was identified a single ORF which showed no relationship to anything present in the database world. Aiming of express each ORF, were constructed expression cassettes in the vector Gateway pK7FWG2. Therefore, the ORFs were amplified by PCR and cloned into a pENTR-TOPO vector. The verification of Construction was performed by sequencing and by an internal digestion with the enzyme EcoRI. Subsequently, the inserts were transferred to the vector pK7FWG2 through a recombination reaction. The confirmation of the presence of the inserts was made by a PCR with specific primers and the constructs also were digested with ScaI to prove that the inserts were inserted into the gateway vector because the TOPO vector does not have this restriction site. The Future prospects are the transfer of the inserts to a model plant, such as tobacco using Agrobacterium and verify if the expression of these recombinant proteins could promote changes in response to saline stress.

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DEEP RNA SEQUENCING REVEALS COLD TOLERANCE MECHANISMS OF INDICA RICE PLANTS DURING EARLY VEGETATIVE STAGE

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Key words: cellulose; cold tolerance; deep sequencing; fatty acid; rice genotypes

The incidence of low temperature during early vegetative stage is one of the major limiting factors to the establishment and development of rice crop. Genes related to cold tolerance in rice have been previously identified, mostly in genotypes from the \textit{japonica} subspecies, which naturally have higher cold tolerance than \textit{indica} genotypes, usually released by research centers in Brazil. To help clarify the physiological and molecular mechanisms that regulate the rice tolerance to cold stress at the early vegetative stage, 100 genotypes were evaluated according to the percentage of plant survival after 10 days at 10°C, allowing the identification of low temperature tolerant and sensitive genotypes from the \textit{indica} subspecies. Comparative transcriptome analysis of 6h cold-treated leaves using high-throughput RNA-seq demonstrated that 3,413 genes (p<0.001), coding for various metabolic pathways including photosynthesis, carbohydrate metabolism, cell wall synthesis, fatty acid unsaturation, detoxification of oxygen reactive species, hormone and Ca\textsuperscript{2+} signaling, exhibited differential expression patterns. Quantitative RT-PCR of selected sequences was used to confirm the high-quality of RNA-seq results. Physiological analyses of cold-treated plants are underway and indicate lower damage to photosynthetic apparatus, higher cellulose content, higher levels of unsaturated fatty acid and higher activity of antioxidant enzymes on the tolerant genotype. Taken together, our data provide evidence that numerous molecular and physiological changes occur during low temperature stress, and that cold tolerance is a complex trait, which can be achieved by several mechanisms.

Supported by FAPERGS, Capes and CNPq
ASSESSMENT OF PHYSIOLOGICAL CHANGES AND TRANSCRIPTOMIC PROFILING IN YOUNG SUGARCANE PLANTS EXPOSED TO WATER STRESS USING RNA-SEQ TECHNOLOGY.

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Keywords: Abiotic stress, gene expression, physiological parameters, RNA-Seq, sugarcane

Sugarcane is a high biomass tropical C4 grass crop which accumulates large quantities of sucrose which is also used for the production of bioethanol, a low-carbon emission fuel. Crop productivity can be significantly impacted by abiotic constraints, especially water availability, because it can severely impair plant growth and performance. Understanding the molecular basis for this loss in productivity will aid in identifying strategies for mitigation. We report here a experiment investigating physiological parameters and transcriptional profiles of genes with acknowledged roles in abiotic stress. Sugarcane cultivar Q208 was grown under controlled temperature and humidity in the Controlled Environment Facility at CSIRO Plant Industry for approx. 40 days after germination and establishment in a temperature-controlled glasshouse. These plants were then subjected to water deprivation for one week. During this period, photosynthesis rate, stomatal conductance, intercellular CO\textsubscript{2} concentration and leaf transpiration were evaluated on the 0, 3\textsuperscript{rd}, 5\textsuperscript{th} and 7\textsuperscript{th} days and the leaves (+1) were collected to measure leaf Relative Water Content and gene expression. RNA-Seq was performed on day 0, day 3 and day 7 leaf +1 control and treatment samples: Illumina 100 bp paired end reads, all samples in one lane. Results of this work indicated that termination of irrigation resulted in measurable physiological effects in young sugarcane plants and analysis of the expression of the chosen stress-response genes revealed significant differential expression between the control and treatment groups. RNA-Seq results revealed transcriptional activity of 29,448 unique genes from sugarcane leaf subjected to water stress. Gene expression analyses in response to water deprivation revealed 68 (early response) and 2,390 (later response) differentially expressed genes on day 3 and day 7, respectively. This will inform further research on water use efficiency in sugarcane, leading to identification of sugarcane varieties with improved tolerance to adverse environmental conditions.

Supporters: FAPESP, CSIRO PLANT INDUSTRY
In Brazil, common bean (Phaseolus vulgaris L.) productivity is severely affected by drought stress due to low technology cultivation systems. Therefore, identifying genes that regulate defense and adaptation mechanisms in plants during the water deficit period is of primary importance. Arbuscular Mycorrhizal Fungi (AMFs) might play an important role in stress tolerance by establishing mutualistic relationships vital for nutrient and water absorption, and regulating several genes as those for aquaporins. A time scale experiment was performed contrasting BAT477 plants (a drought tolerant genotype) inoculated with AMFs (a mixture of Glomus clarum, Acaulospora scrobiculata and Gigaspora rosea) to non-colonized plants, under drought/control, considering different harvesting periods: after 24, 48, 72, 96 and 120h of imposed water deficit. A set of different parameters such as catalase enzymatic activity, leaf’s relative water content, root’s length density and soil moisture were taken in order to validate the water deficit treatments. An in silico search provided nine different ESTs of the MIP group with orthologs to different legumes and Arabidopsis, including four new aquaporins not previously reported for P. vulgaris. Our RT-qPCR assays, involving roots and leaves, suggested the up-regulation of some of these aquaporins during the drought stress response under the influence of the plant-AMF symbiosis: in leaves, MtAQP1 (17.47 fold), PvPIP1;2 (54.17 fold), PvPIP1;3 (21.42 fold), AtPIP2;5 (5.1 fold) and AtPIP2;6 (8.9 fold); and in roots, PvPIP2;3 (14.9 fold), AtPIP2;5 (72.3 fold) e AtPIP2;6 (5.3 fold). The up-regulation of the five transcripts in leaf tissue and the three in roots confirms our initial hypothesis that some genes may have their expression rates increased in the occurrence of the symbiosis. The differential regulations of these genes in leaves of colonized plants also indicate that it may occur under an induction of a signaling cascade that triggers a systemic response affecting the plant as a whole. These genes seem to be strong candidates for breeding strategies aiming to drought tolerance and put AMFs as an important element to that concern.
Plants, during metabolic processes, photosynthesis and respiration, constantly produce reactive oxygen species (ROS). In biotic and abiotic stresses, there is an excessive ROS production. At the same time, the plants have an efficient detoxification mechanism, composed from a set of enzymatic (as catalases, superoxide dismutase and ascorbate peroxidases) and no enzymatic (as ascorbate and glutathione) substances, able to destruct ROS. The flavonoids are also part of the defense system, being important in severe stresses situations. Nevertheless, the imbalance between ROS production and destruction can accumulate those compounds in the plant cell, resulting in the so called oxidative stress. The hormone abscisic acid (ABA) participates of the cell signaling in stresses situations. The aim of this study was to verify the antioxidant system gene expression during ABA treatment of a maize variety with low flavonoid content (P1-ww). Leaves treatments consisted in increasing ABA solutions: control (water), 10, 100 and 1000 µM in three biological replicates. The second leaves were sampled after 3, 6 and 12 hours, then the RNA was isolated and transcript levels were quantified through the reverse transcription quantitative polymerase chain reaction (qRT-PCR) by Delta Ct method. The transcript relative amount of actin (act), Zm actin (Zmact), γ-glutamamylecysteine synthetase (g-ecs), glutathione synthetase (gsh-s), glutathione reductase (gsr1), glutathione S-transferase (gst23), two ascorbate peroxidases (apx1 e apx2), two catalases (cat1 e cat3) and superoxide dismutase (sod2) were related do α-tubulin (tub) transcript levels. The statistical analysis were performed using the Kruskal-Wallis test, 99% confidence and the samples treated with the ABA 1000 µM solution were excluded from the analysis, once presented high variation. It was not observed transcript levels variation for cat3, apx2 and gsr1 in response to ABA treatments. However, we observed significant transcript levels changes of cat1, apx1, sod2, γ-ecs, gsh-s and gst23. The most significant variation was the increase of the transcript levels from the control samples to 100 µM ABA samples. Act, Zmact, cat1 and gst23 transcripts showed increased levels after 3, 6 and 12 hours. Moreover, the same increase occurred to sod2 and γ-ecs transcripts after 6 and 12 hours, for gsh-s transcripts after 3 and 6 hours and for apx1 transcripts after 6 hours. This study, making use of a maize variety with low flavonoid content, will allow to further characterization of the antioxidant system involved in the plant cell responses to ABA.
CHARACTERIZATION OF THE TRANSCRIPTIONAL FACTOR E2F IN RESPONSES TO DNA DAMAGE IN RICE

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Due to their sessile nature and dependence on the light, the plants are under constant contact with factors that affect the integrity of DNA (genotoxins). In response, complex signaling networks are activated to block the effects caused by these stressors. In mammals, the gene family encoding E2F factors have been extensively studied. This family is implicated in various processes, such as cell cycle progression, chromosome segregation and condensation, DNA replication, differentiation and apoptosis. Recent data have established a link between response to double strand breaks (DSB) and activation of E2F target genes, but the involvement of E2F in the signaling of DNA repair remains unknown. The aim of this study was to identify and characterize the rice E2F transcription factor family in response to genotoxic damage. Experiments of treatment of rice plants with UV-B and MMS (Methyl Methanesulfonate) were conducted. In addition, phylogenetic analyzes of this gene family in plants were performed. In rice, previous studies of our group identified six genes belonging to the E2F family, four typical and two atypical E2F. In response to UV light, two of four typical E2F genes and the two atypical E2F genes increased their relative expression after stress compared to the plants grown in control condition. In this experiment, in addition to the E2F and repair genes, we have also analyzed the transcript levels of genes known to be involved with cell cycle and apoptosis, in order to verify if the increasing in E2F gene expression, in response to the stress, is actually related to the response to DNA damage, rather than to the other two cellular processes. To confirm the results, experiments with MMS are currently being conducted. Preliminary results indicate that, in seedlings of 4 weeks exposed for 12 hours to 2,5 mM of MMS, all genes related to cell cycle and apoptosis have a decrease in their expression. On the other hand, some of the members of the E2F family respond by increasing their expression. Phylogenetic analysis revealed that one of these two typical E2F, which showed differential expression, probably acts as a transcription repressor. This statement is based on comparisons with sequences from Arabidopsis thaliana. These results suggest that some members of the E2F family of rice are involved with responses to DNA damage.

Orgão financiador: CAPES, CNPq
The detrimental impact on environment caused by intensification and expansion of the modern agriculture is a major global concern. Current high rates of nitrogen (N) input remains inevitable to achieve a substantial crop production required by the world demand for food and bioenergy. Therefore, nitrogen use efficient crops are a future challenge. The selection of productive genotypes that can perform well under low N availability is essential to elucidate physiological and molecular regulation of nitrogen use efficiency (NUE). To gain further insight in NUE in sugarcane (Saccharum spp.), 22 cultivars were screened under N-sufficient nutrient solution for three months and subjected to short-term influx studies with doubled labeled 15N-ammonium nitrate to determine variation in N uptake efficiency under limited and ample N supply. Estimated NUE as the ratio between biomass and N concentrations revealed a genetic natural variation among the screened sugarcane genotypes. Two contrasting genotypes for NUE were selected. Whilst IAC873396 showed high biomass accumulation and NUE values under both N treatments, IACSP 962042 displayed an opposite phenotype. Short-term influx of 15N-double labeled ammonium nitrate into sugarcane roots of both genotypes showed no significant differences under N-sufficient growth conditions. During N-deficiency, IAC873396 roots presented 44% higher 15N-ammonium nitrate uptake compared to IACSP 962042. These results indicate that at low N inputs an increased N uptake might contribute to high NUE values showed by IAC873396 genotype. To assess the molecular basis of N uptake-related genes involved in NUE, the majors AMMONIUM TRANSPORTERS (AMTs) and NITRATE TRANSPORTERS (NRTs) were identified in sugarcane EST genome. Similar expression levels for ammonium transporter genes were found between both sugarcane genotypes under N-replete growth condition. However, IAC873396 roots presented transcriptional upregulation of ScAMT1;1, ScAMT1;3, ScAMT2;1, ScAMT2;3 and ScAMT3;1 compared to IACSP 962042. The increased AMTs expression levels were followed by the 15% higher ammonium uptake rates in the IAC873396 than IACSP96 2042 roots. In addition, the two main transporters responsible for nitrate acquisition, ScNRT1;1 and ScNRT2;1 showed increased expression levels during N deficiency for IAC873396 genotype. Thus, these results show that the transcriptional regulation of N-transporters reflect the enhanced root uptake capacity for ammonium nitrate displayed in IAC873396 genotype relative to IACSP 962042. The regulatory mechanism of N acquisition in sugarcane root has a key contribution on NUE components that govern the adaptation to N limited environments.
ANALYSIS OF THE EXPRESSION OF THE RESVERATROL SYNTHASE GENE AND THE PRODUCTION OF RESVERATROL IN ARACHIS SPP.

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Key words: resveratrol, resveratrol sintase, Arachis spp., qRT-PCR, HPLC

Resveratrol is a phytoalexin produced under biotic and abiotic stresses and has been found in a limited number of plant species, including peanut (Arachis hypogaea) and its wild relatives. This phytochemical has antifungal, antibacterial and antioxidant characteristics and it is a promising cardioprotective, antitumour and neuroprotective agent. Peanut is an allotetraploid species with an AABB genomic constitution and A. ipaënsis and A. duranensis are the putative donors of the B genome and the A genome, respectively. In comparison to cultivated peanut, diploid wild species are more resistant to fungal and nematode diseases, suggesting a higher level of synthesis of resveratrol. The study of the expression of Resveratrol Synthase (RS) and its consequence in resveratrol production in response to ultraviolet (UV) in peanut and its wild relatives is relevant for the understanding of the resveratrol pathway. In this work, we investigated the expression of the nine RS isoforms and resveratrol production in A. duranensis, A. ipaënsis, A. hypogaea (cv. IAC-Runner-886) and in a synthetic amphidiploid derived from these two wild species. Leaves of 15 plants were collected and exposed to UV for two hours and thirty minutes. Untreated leaves were also collected for the control group and three biological replicates were produced. RT-qPCR and HPLC assays were conducted to evaluate the levels of RS expression and resveratrol production, respectively. The results demonstrated that diploid wild species A. duranensis and A. ipaënsis expressed and produced significantly more RS and resveratrol (around 45 fold than control and 300µg/g respectively), when compared to A. hypogaea and to the synthetic amphidiploid (around 11 fold than control and 200µg/g, respectively). Therefore, wild species could be an excellent source of alleles for a higher resveratrol production in the cultivated peanut for improvement of resistance to abiotic and biotic stresses as well as increase its nutritional value.

Financial support: Embrapa CENARGEN; CAPES, CNPq
IDENTIFICATION OF CANDIDATE GENES ASSOCIATED WITH DROUGHT TOLERANCE IN SORGHUM

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Key words: RNASeq, cDNA library, differential expression, up-regulation, drought stress.

Sorghum is one of the most adapted cereal to water stress. However, drought stress is still a major factor in reducing production in this crop. The development of sorghum cultivars tolerant to water stress is part of the objectives of the Breeding Program at Embrapa Maize and Sorghum. To optimize this process, it is essential to identify genes in drought tolerant genotypes that respond to changes in soil water. Functional genomic tools have enabled large-scale gene expression studies to an unprecedented speed and accuracy, which may lead to the identification of genes involved in differential responses between genotypes under different stress conditions. The objective of this study was to compare gene expression of a drought tolerant sorghum genotype in the presence and absence of the stress to identify candidate gene association with this phenotype in sorghum. The plant material used was an Embrapa Maize and Sorghum 9910032 sorghum genotype that is drought tolerant. Total RNA was extracted from roots of plants under conditions of normal irrigation (100%) and stressed (50%) with two biological replicates per treatment. The cDNA libraries were constructed and sequenced by the company Eurofins (Alabama, USA). The differential expression analysis was performed using the GeneSifter * Analysis Edition software (Perkin Elmer). From this analysis, it was possible to identify 662 differentially expressed genes, of which 21 were up-regulated under conditions of water stress. The increase of expression varied between 10 and 65X. The proteins encoded by these genes can be grouped into three functional groups: i) protein that lacks functional annotation ii) protein post-translational modification, and iii) proteins in response to stress. Now, efforts should be used for the functional characterization and validation of candidate genes that could be used to obtain elite sorghum inbred lines tolerant to drought.

Financial Support: CNPq, Fapemig, Embrapa
The development of cultivars tolerant to limited water resources is a sustainable alternative to mitigate the negative impacts of global climate change. In Brazil, 14.8% of the area planted to maize is affected by drought, which means losses of more than 3.7 million tons of grain. Examples of successful generation drought-tolerant cultivars through marker assisted selection or genetic modification are limited, indicating the need for multidisciplinary efforts in these areas. An important advance in understanding the genetic bases of drought tolerance was the identification of the gene encoding the transcription factor \textit{dreb2A} (Dehydration Responsive Element Binding Protein) from \textit{Arabidopsis thaliana}. Subsequently, the transcription factor \textit{dreb2A} (\textit{Zmdreb2A}) was also isolated from maize (\textit{Zea mays}). This gene is a transcription DREB2A-type whose expression is induced by cold, dehydration, high salinity, and heat stress in maize seedlings. According to literature \textit{A. thaliana} plants expressing the construct \textit{35S::Zmdreb2A} showed growth retardation and tolerance to drought stress. To minimize the negative effect on plant growth, the stress-inducible \textit{RD29A} promoter was used to control the expression of \textit{Zmdreb2A}. Almost all the \textit{RD29A::Zmdreb2A} plants grew as normally as the wild type. The objective of this work was to transform zygotic embryos of maize HiII with the constructs \textit{Zm::Zmdreb2A} and \textit{Ubi::Zmdreb2A}, \textit{Agrobacterium tumefaciens}-mediated aiming the evaluate the effectiveness of different promoters in the regulation of gene expression of the \textit{dreb} gene. Both promoters were isolated from maize. The promoter of the Ubiquitin (Ubi) directs constitutive expression of genes, while \textit{Zm} promoter is induced by water stress. The embryos infected with \textit{A. tumefaciens} EHA101 containing the gene constructs of interest were transferred to co-cultivation medium for seven days at 20°C. After successive subcultures to culture medium supplemented with different concentrations of the herbicide bialaphos, events containing the genetic construct \textit{Ubi::Zmdreb2A} and \textit{Zm::Zmdreb2A}, respectively, were selected. The confirmation of the transgenic plants was performed by analysis of the insertion of the heterologous gene in the genome and also by studying the expression of heterologous protein in plants generated using the Real Time PCR technique.
THE EFFECT OF TETRAPLOIDIZATION ON THE DROUGHT-RELATED CHARACTER TRANSPIRATION RATE PER LEAF AREA (TR/LA) UNDER VAPOR PRESSURE DEFICIT (VPD) IN WILD ARACHIS, SYNTHETIC ALLOTETRAPLOIDS AND CULTIVATED PEANUT

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Key words: Arachis, wild diploid, allotetraploid, stomatal conductance, vapor pressure deficit (VPD)

Peanut (Arachis hypogaea L.) is one of the most economically important legumes and widely grown in the United States, Asia and Africa. Peanut production also plays a significant role in sustainable agriculture. However, drought limits productivity and little is known about tolerance, a complex character that involves several metabolic pathways. Because it is an allotetraploid species, the difference in ploidy and constant evolution caused genetic distance between the diploid wild ancestors and the cultivars, causing the narrowing of the genetic basis of the primary gene pool. Synthetic allotetraploids produced from wild species of Arachis are important for transferring key alleles. Wild species can be more tolerant to drought, but upon tetraploidization, changes in genetic architecture impact the whole plant physiologic system, thus affecting drought response. As stomatal conductance is directly correlated with water conservation, the objective of this study was to evaluate the effect of tetraploidization of wild Arachis on stomatal conductance, indirectly measured as rate of transpiration/leaf area (TR/AF) under high vapor pressure deficit (VPD). Plants were kept in a greenhouse and the rate of transpiration/leaf area (TR/AF) of 17 genotypes was measured: six synthetic allotetraploids, seven wild diploid parentals and four cultivars. Significant differences were observed on the genotypes regarding TR/AF, especially when VPD was above 3 kPa. In all cases, TR/AF for diploids was lower than for the cultivated peanut, showing higher degree of water conservation. The wild-derived allotetraploids did not follow the patterns of the parentals: the TR/AF was higher, that is, more similar to that of cultivated peanut. For instance, the synthetic allotetraploid [A. batizocoi K9484 x A. stenosperma V10309]4x had the highest TR/AF of all genotypes, including cultivated peanut. This suggests that this characteristic is not conserved and changes upon tetraploidization. We conclude that for the peanut breeding, the use of synthetic allotetraploids for introgression of alleles for tolerance to drought has to be taken with care. Studies will be conducted on back-crossed lines between allotetraploids and cultivated peanut to evaluate the effect of known genomic segments on the stomatal conductance and therefore, the effect of introgression of wild alleles.

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Water stress imparted by drought and temperature severity is the most prevalent abiotic stress that limits plant growth and productivity. Plants respond and adapt to these conditions with an array of biochemical and physiological alterations. Deciphering the mechanisms by which plants perceive environmental signal and its transmission to cellular machinery to activate adaptive responses is of critical importance for the development of rational breeding and transgenic strategies leading to ameliorate stress tolerance in crops. Several studies have already described transcription factors related to plant abiotic stress response using strategies of gene expression analysis at transcription level. But it’s well known that gene expression at transcript level do not often correspond with the changes at protein level. Therefore, investigation of changes in plant proteome is highly important since proteins, unlike transcripts, are direct effectors of plant stress response. Differential-expression proteomics is based on comparison of composition of different proteomes. In this work we present a comparison of proteomes from three different cotton cultivars with contrasting levels of tolerance to drought stress in order to identify transcription factors involved in drought stress response. Leaf crude protein extract from each cotton cultivar under drought stress was fractionated by 1-DE prior to MALDI-TOF-TOF mass spectrometry analysis. We could identify 450 proteins in 20-45 kDa fraction. Eighteen of them are transcription factors with known role in abiotic plant stress response. These three cotton samples were also used for LC-MS direct analysis on ESI-LTQ Orbitrap and we could confirm the expression of some transcription factors exclusively on cotton drought tolerant cultivars.

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ISOLATION AND MOLECULAR CHARACTERIZATION OF A NEW DREB TRANSCRIPTION FACTOR FROM PHASEOLUS VULGARIS

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Key-words: common bean, abiotic stress, gene expression, subcellular localization, DNA-gel shift

DREB (Dehydration Responsive Elements Binding) proteins are transcription factors that activate specific genes involved in tolerance to abiotic stress. To generate new information on the search for drought and other abiotic stresses tolerant varieties, molecular biology and bioinformatics can be applied to identify and characterize genes that control plant defense and adaptation mechanisms to water deprivation, excessive salt and to high/low temperature. Based on publicly available DREB soybean sequences, an in silico study that was carried out to find homologous sequences of DREBs in Phaseolus vulgaris. Three bean ESTs highly similar to a soybean DREB were found and used to assemble a complete ORF containing 1062 bp, which was used for primer design and sequencing. The new sequence was very similar to AtRAP2.4 and named as PvDREB6A, according to phylogenetic analysis. Prediction tools showed that the deduced 354 aa sequence has one copy of the AP2 domain, folding in a three β-sheets and one α-helix structure, and presenting important residues and motifs for DNA contacting and binding specificity. In addition, a chloroplast transit peptide was detected at the 52 N-terminal residue. Binding activity was validated by Electro Mobility Shift Assay (EMSA) and subcellular localization was verified by transient expression of PvDREB6A::GFP in Nicotiana benthamiana. To understand PvDREB6A regulation in common bean, a series of experiments were carried out, followed by gene expression analysis using RT-qPCR. The tolerant variety of P. vulgaris was submitted to drought, salt and cold stress, showing an up-regulation of PvDREB6A in stems and roots under all stresses and a down-regulation in leaves under drought and cold stress. Also, the dynamics of gene regulation was analyzed from 12 to 96 hours of drought stress and after rehydration. In leaves, it was repressed at the first 12 h; then a peak occurred at 48 h, decreasing again with drought severity. In stems, the greater induction in gene expression was verified at 12 h and 96 h; and in roots, gene expression was kept activated during all the analyzed period. Finally, PvDREB6A expression was assessed in three contrasting genotypes (BAT 477, Carioca 80 SH and RAB 96). Opposite patterns were observed for BAT 477 (tolerant) and Carioca 80 SH (susceptible) in leaves and roots. Interestingly, RAB 96 (susceptible) showed the same pattern observed in BAT 477, having a significant decreasing in stems. The comparison of expression of stress-responsive genes, like PvDREB6A, among contrasting genotypes indicates the molecular mechanisms used by the plant to overcome a stress, becoming tolerant to it.

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EXPRESSION PATTERNS OF *EGTIP2*, A TIP2 AQUAPORIN CODING GENE FROM *EUCALYPTUS GRANDIS*

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**Keywords:** aquaporin; promoter; tissue-specificity; abiotic stress, *Eucalyptus*.

Aquaporin has important role in various physiological processes in plants, including growth, development and adaptation to stress. In this context, aquaporin is reported to play relevant roles in plant tolerance to unfavorable drought conditions. In this study, a gene encoding a root-specific tonoplast intrinsic aquaporin from *Eucalyptus grandis* (*EgTIP2*) was investigated. *EgTIP2* promoter region (950 kb) was also cloned and functionally analyzed. Gene responsiveness to dehydration was analyzed using relative quantification of *EgTIP2* expression in roots of *Eucalyptus* plants submitted to PEG treatment. Likewise, tissue-specificity and promoter responsiveness to osmotic stress and ABA were investigated using histochemical and relative quantification of GUS expression in stably transformed *Nicotiana tabacum* SR1 plants. In *Eucalyptus*, *EgTIP2* expression was induced by osmotic stress at 6 and 24 hours after plants exposure to PEG8000. Histochemical analyses of GUS activity revealed reporter expression mainly in the vasculature and in root tips of unstressed transgenic seedlings at different development stages (2, 3 and 4 leaves). Moreover, promoter activity was induced by osmotic stress at 3 and 24 h after seedling exposure to mannitol and mannitol/ABA. In contrast, promoter activity was down-regulated at 3 and 12 h after ABA treatment. These results suggest that, under normal conditions, the investigated promoter drives a preferential expression in vascular tissues. Based in these results, we suggest yet, that the investigated aquaporin might play an important role in stress response in eucalyptus.

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SEARCHING FOR A GENE EXPRESSED IN RICE (ORYZA SATIVA) DURING MAGNAPORTHE ORYZAEE INFECTION AND DROUGHT

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Palavras-chave: abiotic and biotic stress, gene expression, rice blast disease, water deficit, cis-elements

Environmental stresses critically influence rice yields, and pathogen attacks are sometimes the most devastating biotic stress. Rice blast disease, caused by Magnaporthe oryzae, destroys entire fields of rice. Similarly, rice is also subject to drought stress, which can negatively impact plant growth and productivity. Environmental conditions induce the expression of a variety of genes, and the products of these genes are thought to promote stress tolerance, regulate gene expression and activate various pathways. The ERD15 gene is identified by its rapid induction during plant stress signaling in several plant species. The induction of this gene during abiotic and biotic stress responses in rice has not been reported. In this study we used RT-qPCR and cis-element analysis to study the expression of the gene OsERD15 in rice during drought or M. oryzae infection. This gene was rapidly and strongly induced by drought and during incompatible rice and M. oryzae interaction. The results also show that the GT-1, MYB, MYC, ERD1, W-box, GCC-box, LTRE and LTRECOREATCOR15 cis-elements are possibly involved in the expression and regulation of OsERD15 during environmental stress conditions. These results suggest that OsERD15 might be involved in multiple signal transduction pathway represented by drought and M. oryzae infection. Additional experimental investigation is required to understand these results and further elucidate the role of OsERD15 in pathogen and drought stress.

Orgão financiador: CNPq
TRANSCRIPTOME PROFILING IN WILD PEANUT
[ARACHIS DURANENSIS] FOR DISCOVERY OF DROUGHT-
RESPONSIVE GENES

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Keywords: Peanut, Arachis duranensis, drought, genes-candidates.

Peanut, Arachis hypogaea, cultivated tetraploid species, is one of the most widely grown grain legumes in the world due to its high energy value. However, most of peanut species are cultivated in drought-prone areas where its productivity is limited; therefore the development of drought-resistant varieties is a priority. In contrast to peanut, wild diploid relatives are sources of alleles related to disease resistance and adaptation to different environments, which are desirable traits of economic importance. In particular, the wild species, A. duranensis has a high adaptability to water limited conditions. The aim of this study is to identify gene expression profiling in A. duranensis plants subjected to gradual water stress and its control irrigated plants. Leaves and roots were collected at five different points of stress, when changes were observed in the pattern of plant transpiration, and after 30 minutes and 72 hours of rehydration. Total RNA was extracted and sample pools were formed for each treatment, using equal amounts of total RNA from individuals in each collection point and for each tissue. Two cDNA libraries were constructed from total RNA purified from control and stressed pools and sequenced by Roche 454 GS-FLX System with Titanium chemistry. A total of 380,601 reads (average size of 390pb) was generated and 21,714 Unigenes were obtained after clustering and assembly. From the most differentially expressed candidate genes related to abiotic stress 20 genes were selected for validation through RT-qPCR. The majority of these genes showed a significantly positive or negative regulation during stress and after rehydration. All information generated here will be important for the characterization of new wild alleles, gene discovery and development of molecular markers.

Órgãos financiadores: Embrapa, CNPq, FAP-DF
GENE EXPRESSION PROFILING OF CANDIDATE GENES RESPONSIVE TO HYDRIC STRESS IN PEANUT WILD RELATIVES

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Key-words: Hydric stress, Arachis duranensis, RT-qPCR, gene expression

Crops are frequently exposed to environmental stress affecting their growth with adverse effects on plant productivity in cultivated areas. Breeding strategies for increasing crop productivity under water limited conditions are essential and require a better understanding of the mechanisms involved in plant response under hydric stress. Drought-tolerant species have the ability to interconnect a range signals that regulate gene expression during stress allowing their survival. Peanut wild relatives (Arachis spp.) have extensive genetic diversity due to its adaptation to the different environments throughout its evolution and are important source of alleles for resistance to biotic and abiotic stresses for the genetic improvement of cultivated peanut (A. hypogaea). In particular, the wild species A. duranensis has high adaptability to water limited conditions. The aim of this study was to identify and validate the expression profile of candidate genes in A. duranensis that are expressed in response to hydric stress under controlled conditions. The trial design was a randomized block with 26-30 A. duranensis seedlings in hydroponic nutrient Hewitt. 15 day-old seedlings were subjected to hydric stress by nutrient solution withdrawal. Roots were collected after 25, 50, 75, 100, 125 and 150 minutes after stress induction (3-4 plants per point). Plants maintained in nutrient solution were used as controls. Total RNA was extracted from roots and the corresponding cDNA synthesized. The expression of eight candidate genes responsive to hydric stress previously identified by our team (Aquaporin, Auxin-repressed protein (ARP), Cytochrome P450, Dehydrin, Late Embryogenesis abundant (LEA) proteins, Methyltransferase, Nitrilase and Trehalose) was evaluated by RT-qPCR. The results showed that Cytochrome P450, Dehydrin and Trehalose are significantly up-regulated and Aquaporin, ARP, LEA, Methyltransferase and Nitrilase are significantly down-regulated throughout the stress. These data may help to elucidate the response to drought stress in this wild species of Arachis as well as assist in the characterization of new wild alleles, gene discovery and development of molecular markers.

Órgãos financiadores: Embrapa, CNPq, FAP-DF
GENE EXPRESSION AND BIOCHEMICAL ANALYSIS OF THE ENZYMES OF THE ANTIOXIDATIVE SYSTEM OF SUGARCANE (SACCHARUM SPP.) UNDER WATER DEFICIT

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Keywords: drought, antioxidant system, oxidative stress, RT-qPCR, enzymatic activity

Sugarcane cultivars with drought tolerance and high yield are desirable for the rapid expansion of sugarcane cultivation usually occupying marginal lands, e.g., regions characterized by having a prolonged water deficit period. In plants, water deficit stress induces oxidative damage due to the increased production of reactive oxygen species (ROS). Drought tolerance may include mechanisms that allow plants to keep metabolism at regular levels, requiring a robust antioxidative system to inactivate ROS, which includes enzymatic pathways, composed by enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and glutathione S-transferase (GST). The elucidation of molecular mechanisms adopted by sugarcane and the biochemical bases of water deficit tolerance would be relevant for the development of tolerant genotypes, while contributing to a reduction in water requirement for irrigation.

Two genotypes selected based on their contrasting behavior in the response to drought were evaluated, where ‘IACPSP94-2094’ was selected as tolerant and ‘IACSP97-7065’ as sensitive to drought. Water deficit was induced by irrigation withdrawn 4 months after planting in greenhouse-grown plants. Samples were collected 7, 14, 20 and 28 days after treatment was imposed. Visual water deficit symptoms were apparent 28 days after treatment, and ‘IACSP94-2094’ exhibited an higher activity of SOD and APX in comparison with control plants. SOD activity was lower in stressed ‘IACSP97-7065’ plants, whereas only a slight increase in activity was measured for APX. CAT and GST showed lower activity for both cultivars in all periods of drought treatments if compared to irrigated control, while GR had negligible activity during treatment. The quantitative analysis of reverse transcripts from three SOD genes showed increased expression for to the Mn-SOD1.1 and Cu/Zn-SOD2.1.1 isoenzymes in the tolerant genotype after 20 days of drought, while only after 28 days gene expression of Mn-SOD1.1, Cu/Zn-SOD1.2 and Cu/Zn-SOD3 isoenzymes was increased in the sensitive one. APX isoform did not show increased expression for the tolerant cultivar and for ‘IACSP97-7065’ only APX2.1 isoenzyme showed increased expression for late drought (28 days). Regarding the two CAT isoforms, CAT3.1 showed basal levels of transcripts in ‘IACSP94-2094’ after 14 days of irrigation suspension, following a similar expression pattern in ‘IACSP97-7065’ during the beginning of water deficit (7 days), and late drought (28 days). The enzymes SOD and APX appeared to be involved with the better performance of ‘IACSP94-2094’, which also maintained the level of transcripts from genes encoding enzymes capable of effectively the ROS. ‘IACSP97-7065’ showed an increase in the level of gene transcripts only at late drought, suggesting an inefficient antioxidant system in this genotype.

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MOLECULAR REGULATION OF NITRATE TRANSPORT SYSTEM IN SUGARCANE (SACCHARUM SPP.) ROOTS DURING NITROGEN DEFICIENCY

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Keywords: nitrogen, uptake, gene expression, membrane transporters

Nitrate concentrations in soils can vary several orders of magnitude. As a consequence, plants have evolved a sophisticated mechanism for nitrogen (N) acquisition to coordinate plant development and biomass production according to N availability. The root system is the primary site to sense nitrate fluctuations and therefore to modulate N uptake system to achieve an efficient N remobilization from the rhizosphere. Compared to other crops, sugarcane (Saccharum spp.) ability to use nitrate as N source is reduced. Therefore, a molecular characterization of nitrate acquisition in sugarcane roots is relevant to improve nitrogen use efficiency in this species. Here we report the physiological and the molecular characterization of nitrate transport in sugarcane N-starved roots. Long-term uptake studies with 15N-labeled nitrate were performed in three-month-old sugarcane plants grown in N replete nutrient solution. When submitted to N starvation, post-labeling 1 d plants showed similar 15N concentrations in roots and shoots compared to N sufficient grown plants. Limitation of N supply for 5d resulted similar accumulation of 15N-nitrate derived in roots and shoots. In contrast, in N-replete conditions, 68% of 15N concentrations were present in shoot while only 32% were shown in roots. These results indicate that root N demand is higher during N deficiency and therefore root to shoot N transport is impaired. To understand the molecular bases of nitrate transport in roots during N deficiency, members of multigene family of NITRATE TRANSPORTERS/PEPTIDE TRANSPORTERS (NRT/PTR) were identified in the sugarcane-expressed genome present in SUCEST database. The quantitative transcript analysis during N limitation growth conditions showed increased expression levels for ScNRT1;1, ScNRT1;2 and ScNRT2;1 which are the major membrane transporters responsible for low and high affinity nitrate uptake in roots. The xylem loader ScNRT1;5 displayed no regulation by N starvation however an upregulation of the xylem unloader gene ScNRT1;8 in N limited roots was observed. Altogether, these results suggest that during N deficiency an increased uptake in roots and a reduced nitrate translocation to shoot are processes dependent on nitrate transporters activity. A coordinated regulation of nitrate transporters might be required to maintain nitrate homeostasis in sugarcane roots thereby ensuring sufficient N supply for root growth in order to exploit the soil solution when N is scarce.

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PHYTOTOXICITY AND LIPID PEROXIDATION INDUCED BY PARAQUAT IN SUGARCANE CULTIVARS


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Keywords: Cultivars, pesticides, resistance, stress

The sugarcane agribusiness in Brazil represents about 3.5% of GDP and the whole production is intended for the sugar and ethanol industries, besides the potential for biomass. The use of herbicides is essential, but may cause severe phytotoxicity, reducing the yield and end product quality. Sugarcane cultivars shown differential tolerance to herbicides; therefore the development of new technologies to accurately and early identifying cultivars that present tolerance to herbicides could help the sugarcane breeding programs. Due the oxidative stress conditions caused by herbicide applications, should occur lipid peroxidation, high phytotoxicity, and cell death. This work aims to correlate visual phytotoxicity and lipid peroxidation with the differential responses of sugarcane cultivars to paraquat herbicide. According to the visual phytotoxicity after 48 hours of paraquat application, the group of plants that showed higher phenotypic damages was represented by the cultivars “IACSP94-2094”, and “SP87-396”, and the “SES” showed irreversible damage leading to plant death. In the group of plants that had low injuries are present the cultivars “SP80-3280”, “SP80-1842” and “RB85-5113”. According to chlorophyll content 48 hours after treatment, the cultivars “SP80-3280”, “Muntok Java” and “CTC-3” showed the highest values while “IAC94-2094”, “SP87-365” and “CTC-1” showed the lowest values. The lipid peroxidation values indicated that 48 hours after paraquat application, the group of plants that shown the highest levels was represented by “CTC-2”, “CTC-5” and “SP89-1115” cultivars. In the group of plants that had low peroxidation is presented by “SP80-3280”, “RB85-5113” and “CTC-2”. We observed that the “SP80-3280” and “SP87-365” cultivars showed differential responses to lipid peroxidation, phytotoxicity and chlorophyll content treatments. The sugarcane “SP80-3280” cultivar showed to be one of the most tolerant to paraquat compared to “SP87-365”.

Financial Support: Cnpq
SALT STRESS INDUCES EXPRESSION OF GENES INVOLVED IN METABOLISM AND CATABOLISM OF ABSCISIC ACID IN STRAWBERRY FRUITS.

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Palavras-chave: Fragaria ananassa, salt stress, ABA, gene expression, hormone response

Strawberries fruits have high consumer acceptability around the world, and is also characterized for its nutritional and functional compounds of interest, that may be influenced by different stresses during crop development. To date, we still lack valuable information on the molecular events that control strawberry fruit adaptation to environmental stresses. This is a complex process involving the coordinated regulation of genes and biochemical pathways in order to keep the metabolism balance through strategies of tolerance or acclimatization. The plant hormone ABA regulates many key processes in plants and serves as an endogenous messenger in biotic and abiotic stress responses. In this study, we evaluated the expression of genes related to osmotic stress response in strawberry fruits: NCED, key gene for ABA synthesis; CYP, key gene for ABA degradation; ASR, a stress response factor. Seedlings of strawberry cv. Camarosa were grown in a greenhouse, distributed in four randomized blocks consisting of six plants each. Doses of 50 mL of 40 mmol (treatment 1) and 80 mmol (treatment 2) of sodium chloride were applied weekly to the soil in order to induce prolonged stress levels in the plants. Throughout the culture development moisture content, soil electrical conductivity as well as photosynthetic parameters were evaluated, which could confirm the phenotype related to stress levels proposed. Ripe fruits were collected and stored at -80 °C. Total RNA samples were isolated with a CTAB-based method and the cDNAs synthesized were used for assessment of gene expression by RT-qPCR. The severe stress (treatment 2) resulted in induction of expression of NCED, a key gene in the ABA synthesis, suggesting that salt stress regulation is dependent of ABA in strawberry fruits. Furthermore, both stress levels induced the expression of CYP707A, probably because ABA is very important for plant growth and stress response, then the homeostasis of endogenous ABA is vital though the balance of metabolism and catabolism. There was no activation of the ABA response factor - ASR due to both salt stress levels. It is suggested that ASR proteins act as a downstream component of ABA and usually, ASR signals shows rapid response, suggesting that the timing of sample collection may have influenced the observed result. Taken together, the results showed that salt stress increased the levels of genes involved in synthesis and catabolism of ABA, suggesting that salt stress response shared ABA signal pathway in strawberry plants. However, quantification of ABA content using chromatography is necessary to further elucidate the results.

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Drought is a significant constraint for the increase of wheat production in Brazilian Cerrado. That abiotic stress causes dramatic reductions in crop productivity and the plant response is known to be a complex mechanism with several different pathways components being up or down regulated. A large number of genes related to drought tolerance mechanisms can be effectively identified in plants using next-generation sequencing (NGS) technology and such information can be useful to plant breeders. In this context, the aim of this work was to analyze the transcriptional profiling, obtained through NGS, in one Brazilian wheat genotype submitted to drought. Seeds of wheat cultivar MGS1 Aliança were grown in glasshouse using pots containing 6.5 kg of soil. Control plants were grown for 5 weeks at 100% of field capacity while, in the drought treatment, plants were watered for 2 weeks at 75% of field capacity following 3 weeks of water deprivation. RNA extraction of pooled leaves or root tissues was carried out with Trizol (Invitrogen). The cDNAs were sequenced using Roche 454 FLX and the data was analyzed using GS De Novo Assembler v2.6. Similarity analysis was obtained using BlastX program and GO (http://www.geneontology.org/) database to obtain sequence annotation. Statistical analyses including control and treatment assembled (isotig) sequences for each set of samples were performed using the DEGseq R method. For leaf samples a total of 619,629 sequences was obtained originating 19,899 isotigs and 31,374 singletons. BLAST results showed that 27% of isotigs sequences are involved in biological processes (most represented by metabolic process), 27% in molecular function (catalytic activity), 22% are cellular components (cell part) and 24% are no hits. For root samples a total of 606,309 sequences was obtained originating 32,085 isotigs and 40,377 singletons. BLAST results showed that 27% of isotigs sequences are involved in biological process (most represented by unclassified sequences); 28% in molecular function (binding); 21% are cellular components (cell part), and 24% are no hits. Statistical analysis showed that 1664 (being 1017 up-regulated) out of 19,899 isotigs in leaves and 2808 (being 1102 up-regulated) out of 32,085 isotigs in roots have statical significance at a p-value <0.001. In leaves 170 out of 1017 isotigs and, in roots, 592 out of 1102 isotigs showed a fold-changed greater than 5. The isotig with lower p-value in leaves was identified as a “photosystem Q(B) protein 2” and in roots was a protein with hydrolase activity, both being up-regulated. The information generated in this study is a valuable resource to identify genes from a Brazilian wheat cultivar, adapted to the Cerrado region, related to drought tolerance. To the best of our knowledge, this is the first report on NGS technology use to study wheat transcriptome in Brazil.

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HOW WILL SUGAR CANE PLANTS RESPOND TO ELEVATED [CO₂] AND DROUGHT STRESS IN THE FUTURE?

Amanda P. de Souza, Thomas Degenkolbe, Lothar Willmitzer & Marcos Buckeridge

Due the Global Climate Change, an increase in atmospheric [CO₂] is predicted with changes in water distribution and temperature. Understanding how plants will respond to those conditions will allow the development of strategies to face this future environment. Sugarcane is a C₄ plant that has a great importance in bioenergy sector, especially in Brazil. Usually C₄ plants do not respond well to elevated CO₂ unless they are cultivated under drought stress. However, sugarcane grown in elevated CO₂ presented an increase of 30% in photosynthesis and an increase of 40% biomass even without imposed water stress. The increase in photosynthesis was related with an increase in electron transport rate as well as genes and proteins associated with this phenomenon. One of the main barriers for expansion of sugarcane culture in order to supply the bioenergy needs is the drought stress. Sugarcane plants grown under elevated CO₂ and water stress maintain higher photosynthesis rate, what leads to an increase in biomass. However, the pathways of these responses are unknown. To understand how these responses are regulated and how elevated CO₂ could improve the plant performance under water stress, we sampled leaves, culm and roots of sugarcane in four different conditions: a) ambient CO₂; b) elevated CO₂; c) ambient CO₂ + drought stress; d) elevated CO₂ + drought stress; during plant development. For each time point, we analyzed a series of physiological parameters and the metabolic profile by GC-MS-TOF. We are also using techniques of next generation sequencing (RNAseq) to identify target genes responsible for the observed responses. To integrate the results from different scales (physiology, metabolomics and transcriptomics), we are using a system biology approach.
EXPRESSION ANALYSIS OF ARABIDOPSIS THALIANA EXPOSED TO HYDROCARBON MIXTURE SHEDS LIGHT ON THE COMPLEX ABIOTIC STRESS RESPONSE PATHWAYS IN PLANTS

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Keywords: Arabidopsis, oil stress, cis-elements, microarray, EGAN analysis

Oil exploitation, extraction, and processing are necessary procedures for our economy, due to oil’s important role in several aspects of human activities. Thus, environmental pollution and risk of oil spills are constant and growing concerns. Mangrove flora is one of the most affected biosystems by petroleum spills, but there is still a lack of studies in the literature analyzing the possible damage or impacts of such contaminants. With the goal of elucidating plant molecular responses to the exposure to complex mixture of hydrocarbons, global gene expression analysis of 10 days-old Arabidopsis thaliana exposed to water soluble fraction of the marine oil MF-380 (WSF-380) was carried out by a high resolution time-course DNA microarray experiment. The transcriptional factors and effector genes related to these pathways were subsequently validated by qPCR. The experiment revealed 340 genes modulated by the stress condition, which were classified into 12 clusters according to their expression profile. Different classes of biological processes were over-represented, such as response to hypoxia, response to osmotic stress, response to oxidative stress and response to heat. The activation of different stress response pathways may be an evidence of the complex chemical composition of the oil, and the great developmental constraint it may represent to plants. In order to better understand the regulatory mechanisms involved in the response to WSF-MF380 stress in plants, analysis of cis regulatory elements was conducted in all genes modulated in the microarray experiment. Genes with significant cis elements in their promoter regions, to which modulated transcription factors might bind, were listed and their expression patterns were further analyzed. Fold change values of the modulated transcription factors of the AP2, AUX-IAA, WRKY, BHLH, MYB and MYB-RELATED families and their possible targets were clustered using the Cluster 3.0v and later visualized in a TreeView image. Possible WSF-MF380 responses were highlighted by EGAN (Exploratory Gene Association Network), and genes, including transcription factors and their targets, were checked by qPCR analysis. The data analysis will help to elucidate the role of those genes in the physiological and molecular response to hydrocarbon derived mixtures stress, such as oil and its derivatives.

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Tissue-specificity and stress responsiveness of the promoter region of a gene encoding an alanine-glyoxylate aminotransferase from *Eucalyptus grandis*.

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**Keywords:** Promoter region, Abscisic Acid, *Pseudomonas syringae*, *Eucalyptus grandis* and GUS expression

The combination of modern biotechnology to conventional breeding techniques has an enormous potential to increase Eucalyptus productivity and stress tolerance. Among the molecular tools necessary for Eucalyptus improvement through the incorporation of transgenic techniques, the identification and characterization of organ-specific or stimulus-dependent promoters is a priority. In this context, the present study aimed to characterize the promoter region of a *Eucalyptus grandis* gene encoding an alanine-glyoxylate aminotransferase (EC 2.6.1.44). Firstly, the tissue-specificity of gene expression was validated in *E. grandis* using quantitative RT-qPCR. Subsequently, an expression cassette containing the 5’-upstream region (1050 bp) of the corresponding gene was fused to GUS and stably transformed in *Arabidopsis thaliana* Col-0 plants by floral dip method. Histochemical analyses of GUS activity revealed reporter expression mainly in the vascular tissues of leaves and roots. Analysis of the promoter sequence using the PlantCare database revealed several regulatory motifs related to hormone regulation and both abiotic and biotic stress responsiveness. Thus, promoter regulation in response to ABA (10 nm) and *Pseudomonas syringae* pv tomato inoculation was further investigated. The results obtained showed a down-regulation of GUS expression in response to ABA and *P. syringae*.

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Identification of genes and promoters related to drought stress in sugarcane and the generation of transgenic plants.

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Keywords: sugarcane, drought, transcriptome, promoter, transgenic.

In Brazil, due to the local and global increasing demand for renewable energy sources, it is notable the expansion of sugarcane cultivation to areas less favorable for cultivation, such as the dry regions of central-western Brazil. Water deficit is one of the main agents that affect plant development. The production of better varieties is critical to the sustainability and expansion of the sugarcane industry. In this way, genes regulated by drought are important targets for obtaining transgenic plants tolerant to stress. Since transgene constitutive expression may cause unwanted effects in transgenic plants, it is also important to identify of stress inducible promoters. The goal of this work is the identification of genes and promoters regulated by drought in sugarcane. We have used different microarray platforms: SUCAST (contains 1,830 genes related to signal transduction), SUCAMET (contains 4,594 genes related to metabolism) and CaneRegNet (contains probes for the identification of sense and antisense transcripts that correspond to 14,522 unique genes). In these platforms we analyzed gene expression of four different sugarcane varieties, two tolerant and two sensitive to drought, after 24, 72 and 120 h of water deficit. From the genes differentially expressed (some transcribed by the sense and/or antisense strand), 53 were selected for qPCR validation. Promoter regions of four genes were identified by Chromosome Walking, selection and sequencing of BACs and the analyses of Whole Genome Sequencing contigs. Seven promoter sequences were selected and cloned into a vector containing the gus reporter gene. Promoter activity was tested and verified through the transient transformation of sugarcane embryogenic calli. For sugarcane transgenics production we selected a drought-induced gene. Through the transformation of sugarcane embryonic calli with a vector for gene silencing we obtained transformed plants with the gene expression reduced. The transcriptome of transgenic plants was analyzed. In comparison with control plants and in irrigated conditions, we identified 352 differentially expressed transcripts. Transgenic plants with silenced gene and control plants were studied in a pilot drought experiment. Physiological data and morphological observations indicated that one of the transgenic events presented tolerance to drought. The comparison between irrigated and drought plants and between tolerant and no tolerant showed that a large number of genes was differentially regulated.

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Plastid genome-coded genes expression in silico analysis from sugarcane transcriptome

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Key-words: library cDNA, in silico, plastome, Saccharum spp., expression profile

Sugarcane (Saccharum spp.) is a C4 grass with high ability to accumulate sucrose in stems, being used mainly for sugar and ethanol production. In Brazilian northeastern, water deficit and high salinity in soils are main abiotic factors that limit growth and development of this crop, affecting sucrose yields. During both stresses, chloroplast photosynthesis rate is reduced due to lower stomatal conductance and CO₂ assimilation, after stress perception and alterations on gene expression profile. In this work, from sugarcane plastome genes sequences (NC_005878.2), belonging to families psa (photosystem I), psb (photosystem II), pet (cytochrome b₆-f complex), ndh (NAD dehydrogenase) and atp (ATP synthase), it was performed a search for such gene families corresponding ESTs, against TIGR Sugarcane Gene Index dataset (SofGI), through BLASTN, with default parameters and e-value cut-off equal to 10⁻⁵. Plastome-derived transcript clusters detected in SofGI libraries and showing significant similarity to probed genes were grouped according to their in silico expression levels. A total of 41 sugarcane TCs (tentative consensus sequences/clusters) presented similarity to the searched plastome; however, 57% of them had no available expression data in original library, so these could not be included in analysis. The distribution of the remaining TCs among different cDNA libraries (~organs/conditions) was achieved by their normalized expression frequency, resulting in a virtual picture of such genes expression along the plant. As expected, higher expression was observed in leaves: mature in the pSL libraries (psbH, psbD, psbZ, psbM, ndhC, atpH, psbK and rbcL); etiolated in LV1 library (petA); immature in LR1 (petG). Very low expression was detected in inflorescence+seeds FL5 (psbT and psbN), roots RT2 (atpI) and stem bark SB1 library (ndhI). It was verified that genes coding for photosystem II subunits presented high expression in leaves, the main light receivers and photosynthetic process players. Plastid-related gene expression studies in sugarcane favours the identification of genes involved in regulation of chloroplast responses, and may be helpful to establish new targets to sugarcane breeding by photosynthesis marker-assisted selection or genetic transformation of chloroplasts, not yet accomplished in C4 plants.

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