GENE EXPRESSION PROFILE AND CARBON ISOTOPE DISCRIMINATION IN SUGARCANE GENOTYPES UNDER WATER DEFICIT STRESS

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FAPESP Process 2008/57923-5 | Term: May 2009 to Apr 2012

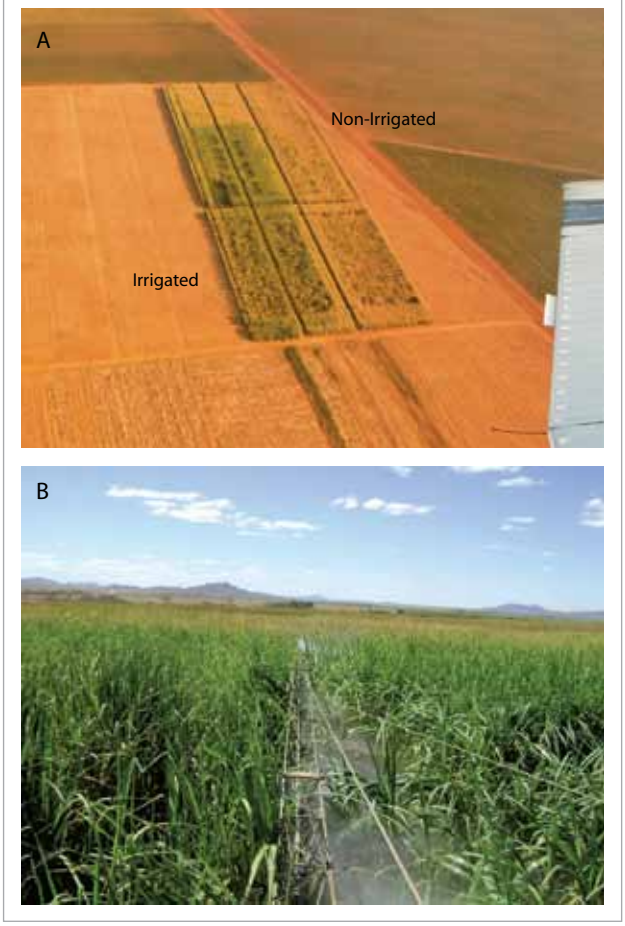

Figure 1 and 2. Field (irrigated and non-irrigated at "Cerrado"). A. Bird's-eye view. B. A general overview of the experiment

Sugarcane (*Saccharum spp.*) is major crop in Brazil as feedstock for the sugar and ethanol industries. To attend the increasing ethanol demand from external and internal markets, the sugarcane industry must expand the cultivated area, incorporating land from 'cerrado' and pastures from Southeast and Western Central Brazil, characterized by a dry winter with a prolonged water deficit period. For the last 10 years, more than 80 sugarcane cultivars have been released in Brazil, but few with yield potential to be cultivated in drought-prone environments. Mechanisms of response and tolerance to water stress have been investigated in model plant species, whose genes were classified into two groups: one includes proteins that act directly on dehydration tolerance, and the other comprises regulatory genes. Previous work on sugarcane response to water deficit stress detected similar induced regulatory genes to the ones from rice and Arabidopsis, but structural genes associated with stress response have not been evaluated. Elucidation of sugarcane mechanisms involved in tolerance to water deficit would be valuable to develop cultivars productive and adapted to drought-prone regions, which could potentially assist in the sustainability of the sugarcane industry in these marginal regions. This proposal intends to establish an efficient and dependable method to evaluate water deficit stress in sugarcane by evaluation of several protocols, to enable the analysis of gene expression profiles between genotypes tolerant or susceptible to water stress using microarrays, followed by validation of differential gene expression by quantitative amplification of reversed transcripts (RT-qPCR). Analyses of marker gene expression (drought- or ABA-related structural or regulatory genes) will be conducted using RT-qPCR to validate the observed physiological responses. At the same time, ^{13}C discrimination technique (Δ) will be tested and optimized to evaluate the genetic diversity available for the trait, together with biochemical and physiological measurements, associated with water use efficiency and, consequently, water stress tolerance.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our work has focused on two sugarcane cultivars developed by the "Instituto Agronômico de Campinas" (IAC), contrasting for water deficit tolerance, selected under field conditions at drought-prone regions of the 'Cerrado' (*Fig. 2*). These contrasting cultivars were used to investigate physiological and molecular mechanisms involved in field tolerance to water deficit through phenotyping and gene expression profiling. Based on physiological evaluations from field and greenhouse experiments (*Fig. 2-3*), the tolerant cultivar showed enhanced features for drought tolerance, such as early stomatal closure; maintenance of leaf water potential; and superior photochemical activity, traits absent in the sensitive cultivar. The tolerant cultivar showed a higher biomass yield at the second year of planting. Changes in ^{13}C discrimination enabled the differentiation among cultivars contrasting for drought tolerance. We have evaluated early screening methods by exposing plants to 15% polyethylene glycol (PEG) in nutrient solution to simulate drought-response, or by evaluating electrolyte leakage after leaf segments exposure to methyl-viologen (Paraquat). Differences in leaf water potential and gas exchange traits were apparent after 72 h between the contrasting cultivars grown on nutrient solution containing 15% PEG. Similarly, the cultivars differed for electrolyte leakage after leaf-disk treatment with methyl-viologen, suggesting the potential application of this approach for early selection for water deficit tolerance, possibly associated with a better performance of the tolerant cultivar against oxidative stress.

To identify genes involved in drought tolerance, leaf samples from the contrasting cultivars grown under irrigated and non-irrigated conditions were analyzed for global expression by microarray and RNA sequencing. From the microarray analysis using a chip with 14,522 genes, 91 (~0.63%) were differentially expressed between irrigated and non-irrigated treatment at early stage of drought, while 576 (~4%) were differentially expressed during severe drought, from which 438 were differentially expressed between genotypes, including many genes traditionally associated with drought response, such as aquaporins, dehydrins, anti-oxidative enzymes, transcription factors (DREBs, ABREs, MYBs), together with novel ones. The RNAseq analysis, using a reference dataset with 43.141 sugarcane genes, revealed 2,300 (5.3%) genes differentially expressed between the tolerant and sensitive cultivars. A high correlation (0.78) was observed between differentially expressed genes identified by microarrays and RNAseq analyses. Typical drought-responsive, together with novel genes, were detected and may play a role in drought tolerance, by shutting down growth while maintaining water potential during drought. We detected a potential novel mechanism for drought tolerance through the differential up-regulation of genes associated with photosynthesis in the tolerant cultivar. Further, genes coding for enzymes controlling oxidative stress appeared to be important during adaptation to water deficit. Differentially expressed genes detected by microarray and RNAseq were validated through qPCR. Identified mechanisms will be tested on cultivars contrasting for water deficit tolerance. Gene functional assays will be conducted in sugarcane and other model system (such as rice) to develop new drought-tolerant cultivars, either by conventional or molecular breeding. The conditions optimized to quickly simulate water deficit by PEG treatment may be used to early screen for drought tolerance.

Figure 3. Greenhouse trial. Instituto Agronômico de Campinas (IAC)

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