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

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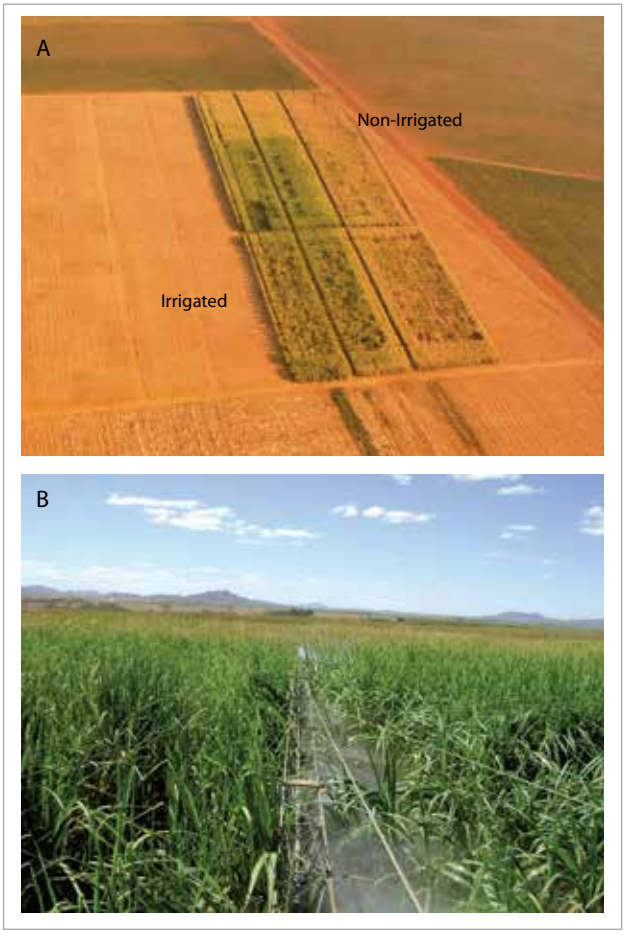

Figure 1. Field (irrigated and non-irrigated at "Cerrado"). A. Bird's-eye view. B. A general overview of the experiment

Sugarcane (*Saccharum spp.*) is major crop in Brazil as feedstock for the sugar and ethanol industries. To attend the increasing ethanol demand, the sugarcane industry must expand the cultivated area, incorporating land from 'cerrado' and pastures, characterized by a dry winter with a long water deficit period. For the last 10 years, more than 80 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 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, promoting 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 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

The Brazilian sugarcane industry is expanding rapidly, particularly to drought-prone regions. We characterized tolerance by phenotyping and attempted to identify genes with roles in drought tolerance. From 100 genotypes, 10 were selected and evaluated for drought tolerance in a field trial in Goianésia and under greenhouse conditions. Based on morphophysiological evaluations, we identified two contrasting genotypes for response to drought stress: 'IACSP94-2094' showed enhanced features of drought tolerance (lower transpiration; maintenance of leaf water potential; and superior photosynthesis activity), limited in 'IACSP97-7065'. Gas exchange and photochemical parameters, carbon isotope discrimination ($\Delta^{13}C$), leakage of CO_2 (φ), and the enzymatic pathway involved in the transport of the C_4 acids from mesophyll to bundle sheath cells were further investigated in these cultivars. 'IACSP94-2094' displayed higher carbon discrimination and leakage (φ) during water stress. Quantitative gene expression profile and enzymatic activity consistently suggested the occurrence of the phosphoenolpyruvate carboxykinase (PCK) alternative during water stress in 'IACSP94-2094'. Leaf samples collected from these contrasting genotypes under irrigation or drought conditions in a field trial at two moments (early and after severe drought) were used to perform microarray analysis. From a set of 14,522 genes, 91 were differentially expressed between irrigated or non-irrigated treatments during early drought, whereas 576 were differentially expressed during severe drought between water treatments, from which 438 were differentially expressed between genotypes. 'IACSP94-2094' showed more changes in expression than 'IACSP97-7065' in genes from pathways associated with drought tolerance, such as oxidation/reduction, hormone metabolism, response to stress, and response to abiotic stimulus by gene ontology analysis. Leaf samples from the same genotypes grown in the greenhouse under similar treatments were used for gene expression profiling by RNAseq. Using the sugarcane assembled sequences as reference (43,141 genes), we identified 2,300 as differentially expressed. There was an important correlation between differentially expressed genes identified by microarrays and observed in RNAseq, with similar expression levels. Differentially expressed genes in 'IACSP94-2094' under stress were associated with photosynthesis, particularly light reaction centers and C4 decarboxylation.

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

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