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

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Figure 1. Overview of a field assay to evaluate cultivars under water deficit conditions of. Photograph kindly provided by the Centro de Cana (IAC), Ribeirão Preto, SP

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 three cultivars selected by the Centro de Cana from the Agronomic Institute of Campinas (IAC) under drought-prone environments, with one clearly more sensitive to drought, while the other two present contrasting behavior to water deficit in terms of biomass yield reduction. These cultivars have been used in various assays to standardize methods to evaluate physiological differences and gene expression associated with imposition of water stress deficit. The procedures evaluated so far included suspension of irrigation; addition of Polyethylene Glycol (PEG) to *in vitro* culture media; and indirect assay with methyl viologen (Paraquat).

The behavior of the three selected cultivars in response to irrigation withdraw under greenhouse conditions confirmed the expected performance obtained from field trials. The susceptible cultivar did not tolerate the 21-day period of exposure to water deficit, while the tolerant cultivar maintained and recovered growth. A similar difference between the tolerant cultivars was observed for the indirect evaluation based on Paraquat treatment. This *in vitro* assay using methyl viologen has been tested as a quick and indirect method to discriminate among genotypes for water deficit tolerance. Difference in tolerance to methyl viologen treatment is evaluated by placing leaf disks on a buffer solution containing this chemical (*Figure 2*), and the conductivity of the solution is evaluated. Differences among genotypes may derive from differences in tolerate oxidative stress. Differences in buffer solution conductivity was observed between cultivars, with large leakage of electrolytes from the sensitive cultivar under treatment with methyl viologen, while the less difference in conductivity was observed for the solution containing leaf disks from the tolerant cultivars. Similar assay has been conducted comparing an old *Saccharum officinarum* cultivar (Muntok Java), supposedly a more drought sensitive genotypes to a wild relative *Saccharum spontaneum* (SES), recognized as more tolerant to water deficit. There was little difference in buffer solution conductivity between both accessions suggesting that this assay might evaluate for only certain mechanisms which could be associated with drought tolerance.

The addition of PEG to culture media *in vitro* or *in vivo* has been adopted as a way to standardize stressful conditions by increasing osmotic potential. An assay to establish a suitable concentration of PEG 8000 to impose water stress indicated that 15% PEG was sufficient in a short period without harming the plants. Plants derived from this assay are being used to investigate the expression pattern of structural genes associated with stress response.

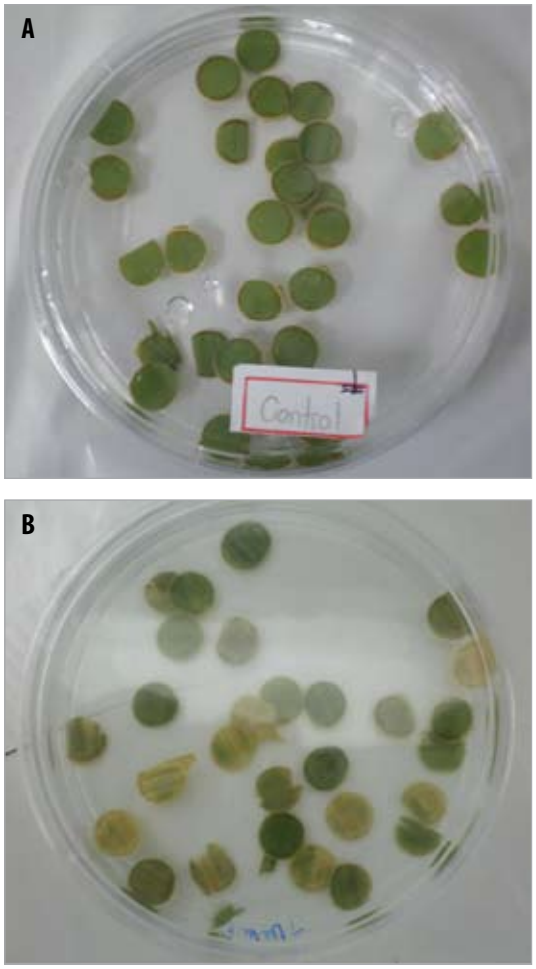

Figure 2. In vitro *assay to establish sensitivity to methyl viologen evaluated by electrolytic conductivity to be correlated with tolerance to water deficit. A) Leaf disks without treatment; B) same cultivar under 3 mM methyl viologen*

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