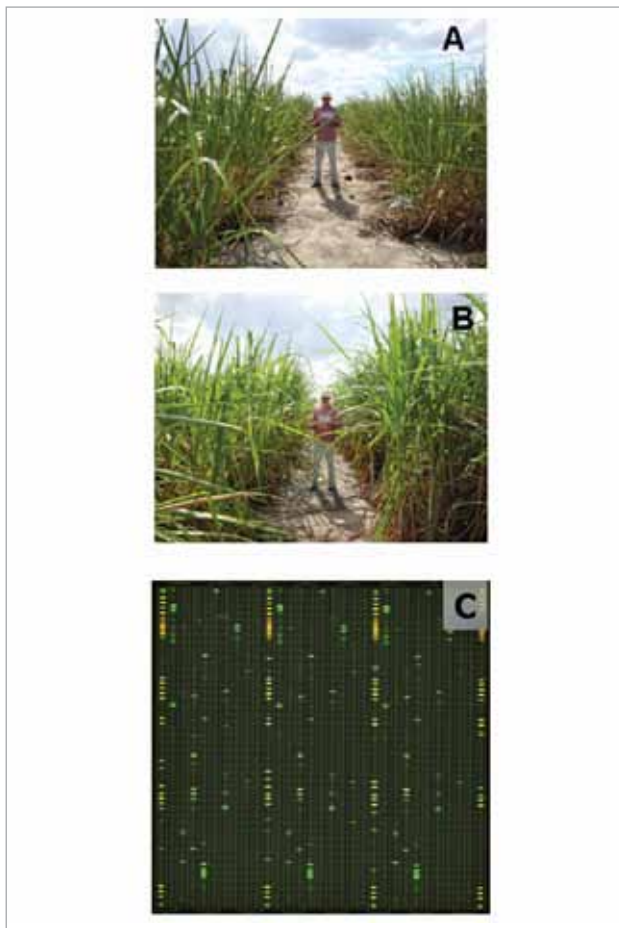


ANALYSES OF DROUGHT TOLERANCE IN SUGARCANE USING TRANSCRIPTOMICS AND METABOLOMICS

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miRNA expression in sugarcane. Plants from RB867515, a drought tolerant variety, were grown for seven months in the field without irrigation (A) or with irrigation (B). RNA was extracted and hybridized with DNA chips (C). Green spots indicate miRNA down-regulated by drought stress, while red spots indicate up-regulated miRNA

We have been working with sugarcane genomics aiming the identification of genes associated to agronomical traits. In this project we aim to identify miRNAs that are modulated by drought stress in sugarcane. To this end we will use genetical genomics, comparing the expression profiles of two groups of sugarcane varieties contrasting for drought tolerance. Each group has two varieties that will be grown in the field, under irrigation or without irrigation. This experiment will capture the plant responses in a real situation of water scarcity. Since field experiments are expected to present high degrees of variation, the same varieties will also be cultivated in greenhouse, in a replicated experiment. These experiments will be conducted by Dr. Laurício Endres (Federal University of Alagoas), with whom we cooperate in another project funded by Research and Projects Financing (FINEP) and National Council for Scientific and Technological Development (CNPq). The miRNA expression will be identified using DNA chips containing all known miRNA and also new sugarcane miRNA that will be identified *in silico* (a second objective of this work). miRNA with interesting expression profiles will be further evaluated by qRT-PCR and *in situ* hybridization. Target genes will be identified *in silico* and validated by qRT-PCR. The metabolome of same plants used for miRNA analysis will also be evaluated by Dr. Marcelo E. Loureiro (Federal University of Viçosa), who is part of a joint project founded by Minas Gerais State Agency for Research and Development (FAPEMIG). A final objective of this project will be the correlation of the expression profiles of miRNA and their targets with the metabolic changes observed in drought-tolerant and drought-sensitive plants.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

To identify sugarcane miRNA *in silico* we developed a strategy that allowed us to extract 100 new candidate miRNA from the SUCEST database. These new miRNA were printed in a customized miRNA chip containing also all known miRNA, named miRCANA, using the arraying services from LC-Sciences (USA). Sugarcane plants grown in the field presented clear effects of drought stress and differences were observed between drought tolerant and drought sensitive varieties. The hybridization of miRCANA chips was done using RNA from 7 months-old plants grown without or with irrigation. Data evaluation allowed the identification of 12 miRNAs that are associated to drought stress tolerance in sugarcane. The validation of chip data using qRT-PCR from other biological replicates is underway. This will allow us to identify the truly positive miRNA. The experiment with sugarcane plants growing in greenhouse will be finished soon and we expect that it will allow us to obtain data with less variability. It will be interesting to compare the expression profiles in plants grown under field and greenhouse.

MAIN PUBLICATIONS

This is the first year of the project and no publication was produced so far. However, a list of recent publications from our group related to sugarcane genomics is shown.

Drummond RD, Pinheiro A, Rocha CS, Menossi M. 2005. ISER: Selection of differentially expressed genes from DNA array data by nonlinear data transformations and local fitting. *Bioinformatics*. **21**:4427-4429.

Vicentini R, Menossi M. 2007. TISs-ST: A web server to evaluate polymorphic translation initiation sites and their reflections on the secretory targets. *BMC Bioinformatics*. **8**:160.

Menossi M, Silva-Filho MC, Vincentz M, Van-Sluys MA, Souza GM. 2008. Sugarcane Functional Genomics: gene discovery for agronomic trait development. *Int. J. Plant Genom.* **2008**:458732.

Vicentini R, Menossi M. 2009. The predicted subcellular localisation of the sugarcane proteome. *Funct. Plant Biol.* **36**:242-250.

Vicentini R, Felix JM, Dornelas MC, Menossi M. 2009. Characterization of a sugarcane (*Saccharum spp.*) gene homolog to brassinosteroid insensitive1-associated receptor kinase 1 that is associated to sugar content. *Plant Cell Rep.* **28**:481-491.

Gentile A, Ditt RF, Dias FO, Silva MJ, Dornelas MC, Menossi M. 2009. Characterization of ScMat1, a putative TFIIH subunit from sugarcane. *Plant Cell Rep.* **28**:663-72.

Papini-Terzi FS, Rocha FR, Vêncio RZ, Felix JM, Branco DS, Waclawovsky AJ, Del Bem LE, Lembke CG, Costa MD, Nishiyama MY Jr, Vicentini R, Vincentz MG, Ulian EC, Menossi M, Souza GM. 2009. Sugarcane genes associated with sucrose content. *BMC Genomics*. **10**:Article Number:120.

Felix JM, Papini-Terzi FS, Rocha FR, Vêncio RZN, Vicentini R, Nishiyama-Jr MY, Ulian EC, Souza GM, Menossi M. 2009. Expression profile of signal transduction components in a sugarcane population segregating for sugar content. *Trop. Plant Biol.* **2**:1935-9756.

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