

SUGARCANE SIGNALING AND REGULATORY NETWORKS

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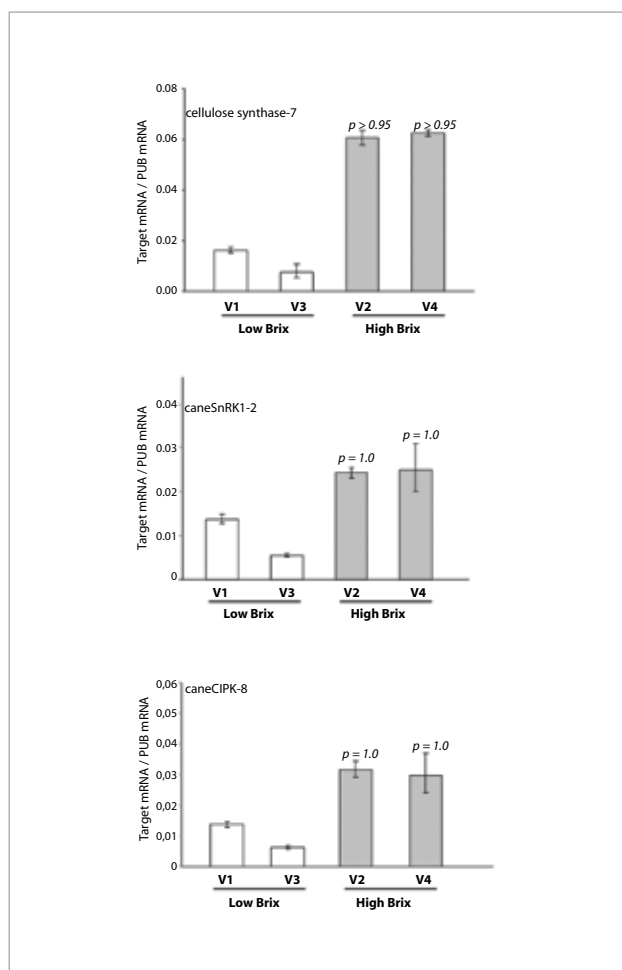


Figure 2. Cell wall metabolism and cell signaling genes associated with yield and sucrose content. Cultivars contrasting for biomass and sugar yield had their transcriptome profiled in search of genes that might be used as biotechnological tools for sugarcane improvement. The Figure shows qPCR experiments for 3 genes in two cultivars that are late in sugar accumulation and low biomass (V1 and V3) and two cultivars that are rich and precocious in sugar and biomass accumulation. (Waclawovsky, A. J., Sato, P. M., Lembke, C. G., Moore, P. H and Souza, G. M. (2010). Sugarcane for Bioenergy Production: an assessment of yield and regulation of sucrose content. Plant Biotechnology Journal. 8:1-14.)

We aim to study signaling and regulatory networks in sugarcane and to develop tools for a systems biology approach in this grass. As a starting point we intend to characterize three agronomical traits of interest: drought, brix and fiber content. We will study gene categories with a well known regulatory role (Transcription Factors, Protein Kinases and Phosphatases), conduct studies on the Transcriptome, produce transgenics, develop a database and computational tools to integrate the several levels of information and we will initiate the whole genome sequencing of a brazilian sugarcane cultivar. In parallel, we intend to implement ChIP-Seq technology in sugarcane, to identify TF targets and gene promoters. The results will have multiple direct consequences on breeding programs that frequently select for CREs and TF changes in search for genotypes better adapted to the environment and with increased agronomical performance. PKs activate signaling cascades in response to environmental stimuli and our studies point to a predominant role of PKs in the regulation of sucrose content and drought responses. To identify new genes associated to brix, drought and fiber content we will characterize the transcriptome of genotypes and cultivars that contrast for these traits using oligonucleotide arrays. Genes of interest will be functionally evaluated by generating transgenics altered for their expression. To integrate the immense amount of public data and that generated by this project a robust computational infrastructure and database will be developed. The SUCEST-FUN database will integrate the gene sequences, promoters, CREs, expression data, agronomical, physiological and biochemical characterization of sugarcane cultivars. We will also participate in the development of the GRASSIUS database to establish sugarcane, rice, maize and sorghum regulatory networks.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Modern sugarcane cultivars are complex hybrids with a giant genome resulting from crosses among several *Saccharum species*. Traditional breeding methods have been employed extensively over the years to develop improved varieties. Currently, commercial yields are at the range of 84 tons/ha/year. Our calculations of sugarcane yield potential lead us to believe that the theoretical maximum would be around 380 tons/ha/year (Table 1). Conventional variety improvement is limited by the narrow pool of suitable genes and the lack of biotechnological tools. Clearly, molecular genetics is seen as promising to assist in the development of improved varieties but a lot has to be done to bring sugarcane in par to other crops in terms of adequate technologies. Our group seeks to associate function to sugarcane genes using a variety of tools, in particular through the study of the sugarcane transcriptome and the production of transgenics. We developed customized oligoarrays representing 14,000 sugarcane genes. The arrays have been used to identify the transcriptome associated to yield, stress responses, sugar and cell wall metabolism, hormonal regulation and the circadian rhythm of sugarcane plants. Overall we have conducted more than 300 hybridizations totaling over 40,000 thousand differential gene expression data points. Bioinformatics tools have been implemented and a database has been created that allow for storage and data mining. The SUCEST-FUN Database (<http://sucest-fun.org>) has been developed in the concept of the mediator approach that incorporates concepts from Data Warehouse and Federation and will allow for heterogeneous data integration. A number of genes are being introduced into sugarcane plants and transgenics. We verified gene function through the generation of plants with increased sucrose content and drought tolerance. The SUCEST-FUN Database assembles different databases such as the sugarcane EST Database (SUCEST), signal transduction, transcription factors and metabolism gene catalogues which include expression data and records of the agronomic, physiological and biochemical characteristics of sugarcane cultivars. We have also started sequencing the genome of a Brazilian cultivar and identified gene promoters. Shot-gun sequencing using the Roche 454 Titanium platform is underway and well as BACs enriched for genes of interest to help assemble this giant genome.

Table 1. Average, maximum and theoretical sugarcane yield and total dry matter production

Type of yield	Cane yield (t ha ⁻¹ yr ⁻¹)	Biomass*	
		(t ha ⁻¹ yr ⁻¹)	(g m ⁻² d ⁻¹)
Commercial Average	84	39	10.7
Commercial maximum	148	69	18.8
Experimental maximum	212	98	27.0
Theoretical maximum	381	177	48.5

* *Plant Biotechnology Journal*. **8**:1-14.

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- Lembke CG, Nishiyama-Jr MY, Sato PM, Andrade RF, Souza GM. Identification of sense and antisense transcripts regulated by drought in sugarcane. *Plant Molecular Biology.* (accepted)
- Begcy K, Mariano ED, Gentile A, Lembke CG, Souza GM, Menossi M. A novel stress-induced sugarcane gene confers tolerance to drought, salt and oxidative stress in transgenic tobacco plants. *PLoS ONE.* (accepted)
- Patent. "Genes associated to sucrose content". USPTO 11/716,262. PCT/BR2007/000282. Souza GM, Papini-Terzi FS, Rocha FR, Waclawovsky AJ, Vêncio RZN, Marques JO, Felix JM, Teixeira MM, Buckeridge M, Souza AP, Ulian EC. Universidade de São Paulo, Universidade de Campinas, FAPESP, Centralcool and CTC.

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