REGULATION OF SUCROSE CONTENT AND YIELD IN SUGARCANE

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Keywords: sugarcane, transcriptome, sucrose, yield, transcriptome, genome sequencing

Introduction

Sugarcane is an important model for studies of source-sink relations due to its ability to store high concentrations of sucrose in the culms. The use of recombinant DNA technology tools as a strategy to raise the concentration of sucrose content in sugarcane requires a broad knowledge related to breeding, physiology, metabolism and regulation associated with sucrose accumulation. The main objective of this study is to understand the behaviour of physiological processes, such as photosynthesis and yield, in high brix genotypes. In the log run we would like to be able to define gene networks associated to agronomic traits of interest in this crop and thus are sequencing the sugarcane genome.

Results

To evaluate the transcriptome associated to sucrose content we have been over the years using cDNA microarrays and oligo arrays and have defined transcription factors, protein kinases, protein phosphatases and receptors that might have a role in the process. Recently we analysed several populations of the RIDESA breeding program grown in the field: *(i)* 4 sets of F1 progeny grown since 2005 (stage T1) as a result of crosses between contrasting parents in relation to soluble solids content (Crossing I: SP83-2847 x TUC71-7; Crossing II: SP70-1143 x RB925211; Crossing III:SP80-3280 x RB855156 and Crossing IV: RB855002 x RB855035) and *(ii)* a T3 progeny derived from two cycles of selection, which aimed to increase the concentration of favorable alleles for brix enrichment. The parameters measured were: brix content, diameter of internode 3 to the last base of stem, plant height, culm length, leaf area and photosynthesis rate. All measurements were done from seven months old field

grown sugarcane plants. Among the data collected, there seems to be a slight trend in the assimilation of CO_2 in relation to brix value (higher brix correlates to a lower rate of carbon dioxide assimilation). However, there are cases where genotypes considered as high brix exhibited greater rates of assimilation and vice versa. This is an interesting finding that might indicate a path towards adding more sucrose on sugarcane by increasing the photosynthetic rate. The correlation between yield and brix also seems to reveal a slight trend in which higher brix is related to greater productivity for some genotypes. Thus, our data seems to indicate that some genotypes seem to have achieved a desensitization of source-sink relationships, where higher brix genotypes showed a higher rate of CO_2 assimilation than those reported for genotypes with lower brix. Previous studies suggest that stored sucrose prevents increases in the rate of CO_2 assimilation in genotypes of high sucrose, through a negative feedback not fully explained yet. This is corroborated by the fact that *Saccharum officinarum*, an ancestor of sugarcane hybrid cultivars that has high sugar content, has lower photosynthetic rates than *Saccharum spontaneum*, which has a low sugar content. Genotypes were expression profiled using oligoarrays. The regulatory pathways leading to activation or deactivation of photosynthesis and sucrose accumulation will be used in the generation of transgenic plants and in search for molecular markers useful for the selection processes.

Also, we are obtaining data on sugarcane promoters using a BAC-by-BAC and a Whole-Genome-Shotgun approach. ChIP-Seq experiments are underway that might contribute to the definition of gene networks selected by breeders in the improvement for high sugar and high yield plants. We chose to sequence a brazilian cultivar, SP80-3280 using WGS. The goal is to obtain 1x coverage of the polyploid genome (or 10 x coverage of the monoploid) and use sorghum as reference to identify promoter regions. So far we have obtained using the Roche 454 System 6 Gbp of sequences (17 million reads). A preliminary assembly using Newbler generated 598 Mb of assembled sequences (776 thousand contigs) that when aligned to sorghum yield in average 91% coverage of genic regions, 44% at 5' gene regions and 68% at 3'. The results confirm the similarity analysis obtained from promoters cloned using *genome walking* that a lower similarity is found at the promoter regions when sugarcane and sorghum are compared while the gene regions are 98% identical.

Data from different sources are being integrated with the aid of the SUCEST-FUN Database (http://sucest-fun.org) using Federation approaches. This platform is being composed of new algorithms and analysis tools, based on mathematical, statistical methodologies and

biological resources that will exploit the whole Sugarcane genome, the Transcriptome and the Functional genomic results that will allow us to employ a Systems Biology approach in Sugarcane to identify regulatory gene networks.

The SUCEST-FUN database assembles different sugarcane databases such as the SUCEST EST Database, gene catalogues such as the SUCAST (Signal Transduction) and the SUCAMET (Metabolism) Catalogues, expression data, genome data, the GRASSIUS database and the agronomical, physiological and biochemical characterization of sugarcane cultivars. Our goal is to study gene expression regulation through the use of tools that will allow a Systems Biology approach in sugarcane.

Conclusions

The group has succesfully identified genes associated to sucrose content and is persuing the construction of gene networks that can aid in our understanding of carbon partition and breeding strategies. The sugarcane genome is being sequenced for the identification of genes and promoters and a integrated database has been made available for the community.

Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP and CNPq.

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