ISOLATION AND IDENTIFICATION OF microRNAS AND TARGETS IN SUGARCANE

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Figure 1. Cover of the Vol.64 of the Journal of Experimental Botany where our paper entitled "Global analysis of the sugarcane microtranscriptome reveals a unique composition of small RNAs associated with axillary bud outgrowth" was cover

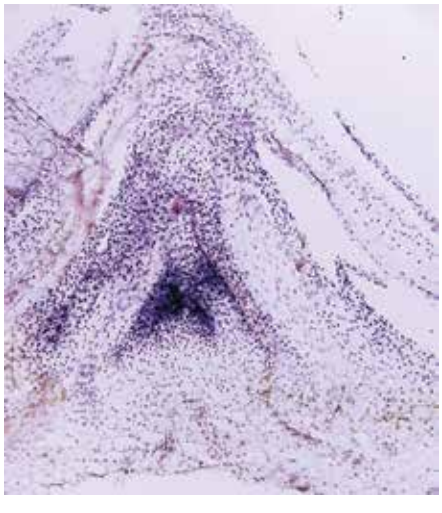

Figure 2. Spatiotemporal expression pattern of the miRNA miR159 in axillary buds of sugarcane. A probe of a 3'-labelled LNA-modified oligonucleotide detecting miR159 was hybridized with longitudinal sections of vegetative buds

Small regulatory RNAs and their targets form complex regulatory networks that control cellular and developmental processes in multicellular organisms. microRNAs (miRNAs) are a growing class of endogenous small RNAs that act in trans to regulate the expression of gene targets. miRNAs are processed from long, noncoding RNA polymerase II-dependent primary transcripts into mature miRNA (~21-24 nucleotides in size). Plant mature miRNAs and their targets frequently show near-perfect complementarity, facilitating their prediction using in silico approaches. Most of the known miRNA target genes are transcription factors that regulate critical steps during plant development.

The combination of cloning, deep sequencing and in silico approaches allows the discovery of conserved and species-specific miRNAs. Such approaches can also identify miRNAs that accumulate in specialized tissues/organs, such as apical and axillary meristems (*Figure 1*) as well as lateral buds. Members of some gene families involved in axillary meristem initiation and its further development are targets for regulation by miRNAs. Furthermore, transgenic and mutant plants overexpressing specific miRNA genes display increased number of branches/tillers as compared to wild-type plants. These findings suggest that miRNAs have important roles in this aspect of the development, which impacts the ultimate plant shoot architecture.

Shoot architecture is an important factor impacting biomass production and management practices for many crops, which are relevant characteristics for attractive biofuel crops. Although shoot architecture is to some extend influenced by environmental factors, it is determined mainly by the plant's genetic program that likely includes the action of miRNAs and their targets. Therefore, the identification and characterization of miRNAs involved in sugarcane plantlet emergence and development would increase our knowledge about the molecular controls of the establishment of plant shoot architecture.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In our first publication, we computationally identified 19 distinct sugarcane miRNA precursors, of which several are highly similar with their sorghum homologs at both nucleotide and secondary structure levels. The accumulation pattern of mature miRNAs varies in organs/tissues from the commercial sugarcane hybrid as well as in its corresponding founder species *S. officinarum* and *S. spontaneum*. Using sugarcane *MIR827* as a query, we found a novel MIR827 precursor in the sorghum genome. Based on our computational tool, a total of 46 potential targets were identified for the 19 sugarcane miRNAs. Several targets for highly conserved miRNAs are transcription factors that play important roles in plant development. Conversely, target genes of lineage-specific miRNAs seem to play roles in diverse physiological processes, such as *SsCBP1*. *SsCBP1* was experimentally confirmed to be a target for the monocot-specific miR528. Our findings support the notion that the regulation of *SsCBP1* by miR528 is shared at least within graminaceous monocots, and this miRNA-based post-transcriptional regulation evolved exclusively within the monocots lineage after the divergence from eudicots.

In our second publication, we employed sRNA next-generation sequencing as well as computational and gene-expression analysis to identify and quantify sRNAs and their targets in vegetative axillary buds of sugarcane. Computational analysis allowed the identification of 26 conserved miRNA families and two putative novel miRNAs, as well as a number of trans-acting small interfering RNAs. sRNAs associated with transposable elements and protein-encoding genes were similarly represented in both inactive and developing bud libraries. Conversely, sequencing and quantitative reverse transcription-PCR results revealed that specific miRNAs were differentially expressed in developing buds, and some correlated negatively with the expression of their targets at specific stages of axillary bud development. For instance, the expression patterns of miR159 and its target *GAMYB* suggested that they may play roles in regulating abscisic acid-signalling pathways during sugarcane bud outgrowth. Our work reveals, for the first time, differences in the composition and expression profiles of diverse sRNAs and targets between inactive and developing vegetative buds that, together with the endogenous balance of specific hormones, may be important in regulating axillary bud outgrowth.

In collaboration with other groups, we mapped sRNAs in the sugarcane genome and its transposable elements (TEs). The results presented support the conclusion that distinct small RNA-regulated pathways in sugarcane target several lineages of TE elements and maybe several sugarcane loci.

MAIN PUBLICATIONS

Zanca AS, Vicentini R, Ortiz-Morea FA, Del Bem LE, da Silva MJ, Vincentz M, Nogueira FTS. 2010. Identification and expression analysis of microRNAs and targets in the biofuel crop sugarcane. *BMC Plant Biol*. 24:10:260.

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