IDENTIFICATION AND CHARACTERIZATION OF microRNAS AND THEIR TARGETS IN SUGARCANE

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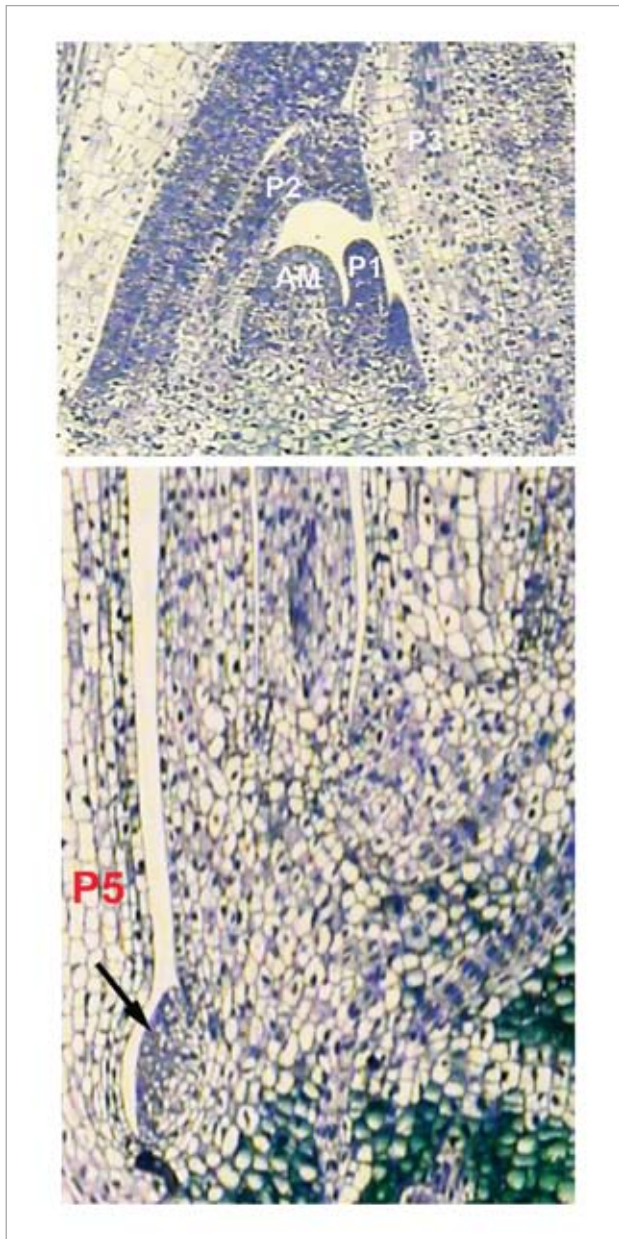Luiz de Queiroz Agriculture School / University of São Paulo (ESALQ/USP)

Figure 1. Meristematic tissues of sugarcane. The top panel shows the vegetative apex of a three-month-old S. officinarum plantlet. The bottom panel shows an axillary meristem (arrow) of the same plantlet. AM: apical meristem; P1 to P5: examples of young leaf primordia

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

MiRNAs have been intensively studied in a wide range of plants over the past few years, but no systematic and comprehensive study has been performed on sugarcane, one of the most promising biofuel crop worldwide. To initiate our research, we searched for conserved sugarcane miRNA precursors using sequencing homology- and secondary structure homology-based strategies. Such strategies allowed us to retrieve several non-redundant miRNA precursors from EST and genomic survey sequence databases. The precursors were classified into 14 families.

Using an in-house BLASTn-based algorithm, we identified more than 40 potential non-redundant target sequences for the 14 sugarcane miRNA families. Consistent with the essential roles of miRNAs in regulating a variety of biological processes in plants, sugarcane target genes seem to be associated not only with development but also with diverse physiological processes. *In vivo* cleavage of some target transcripts was experimentally validated.

We subsequently evaluated the expression of selected miRNAs identified in distinct tissues/organs from commercial sugarcane hybrids as well as from two wild ancient progenitors (*S. officinarum* and *S. spontaneum*). Some miRNAs presented variations in abundance in organs/tissues of the hybrid as well as when comparing the same organs/tissues at similar developmental stages of *S. officinarum* and *S. spontaneum*. It is noteworthy that several tested miRNAs, though at variable levels, are expressed in lateral buds and apical meristems (*Figure 2*).

Functional studies may provide clues on the possible roles of these miRNAs in shoot architecture. We are currently testing this hypothesis by overexpressing microRNA precursors in sugarcane and model plants (such as *Arabidopsis* and *Brachypodium distachyum*). Additionally, we are generating small RNA libraries from dormant and active sugarcane lateral buds. This work would identify novel and species-specific miRNAs and other small regulatory RNAs associated with sugarcane bud outgrowth.

Our Tools
Find Target of Sugarcane miRNAs
Find Target of Sugarcane miRNAs in Sorghum and Rice genome data
Blast against TIGR Gene Indices (external link)

Data of 0-4 mismatches dataset
SOGI all hits in driver database
Predict secondary RNA structure of putative sugarcane pre-miRNAs
111 selected unique gene index cluster

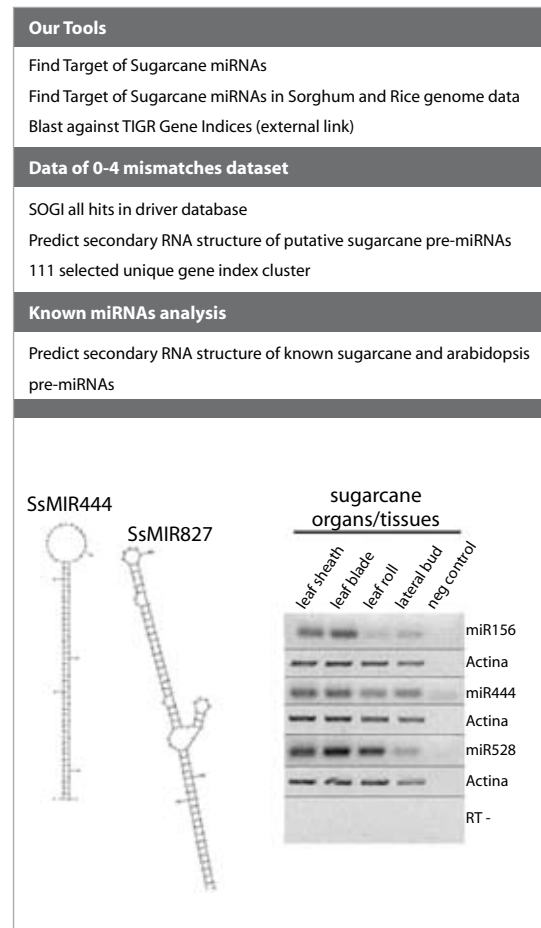| Known miRNAs analysis |
|---|
| Predict secondary RNA structure of known sugarcane and arabidopsis pre-miRNAs |

Figure 2. Sugarcane miRNA database (ScmiRbase). Top panel: web page containing the main search tools of the ScmiRbase. Bottom panels: examples of predicted secondary structures of sugarcane miRNA precursors and expression profiles of mature miRNAs in distinct organs/tissues

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