IDENTIFICATION OF TARGET GENES AND PROMOTERS RELATED TO DROUGHT STRESS AND THEIR ANALYSIS THROUGH THE GENERATION OF SUGARCANE TRANSGENIC PLANTS

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Sugarcane is one of the most important crop plants in the world due to its unique capacity of accumulating high levels of sucrose in its stems. Drought is one of the major stresses that limit normal plant development. Microarrays and oligoarrays are being used by our group to study sugarcane responses to drought. Over the years we developed several plataforms to profile sugarcane gene expression: a SUCAST cDNA array containing 1,879 genes related to signal transduction (Rocha *et al.,* 2007), a SUCAMET cDNA array containing 4,594 genes related to metabolic pathways and an Agilent oligo array with probes in sense and/or antisense orientation, representing 14,522 sugarcane unique genes. Gene expression profiles were evaluated after 24h, 72h and 120h of drought stress in sugarcane cultivars sensitive and tolerant to drought. A total of 217 and 96 differentially expressed genes were identified in SUCAST and SUCAMET arrays, respectively. Using the Agilent array, 830 genes were observed to be differentially expressed including sense and antisense transcripts. qRT-PCR was used to validate some of the gene expression data. A gene related to signal transduction and a gene related to protection against drought were selected for further studies. Transgenic sugarcane plants were obtained. Gene expression was analyzed in PCR-positive plants and we observed that in eight plants the gene was successfully silenced. Physiological differences between silenced and control plants and between different transgenic events were observed when these plants were subjected to drought for seven days. Promoter identification is being conducted for the two genes. BAC clones containing the genes were selected in a sugarcane BAC library. The clones were sequenced using the *454 Genome Sequencer FLX System* (Roche) and the Newbler Software was used for sequence assembly. Regions upstream of the gene initiation and with approximately 1,8 kb were cloned in sequencing vectors. Those regions will be transferred to a vector containing GUS reporter gene and evaluated in transgenic plants.

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