CHARACTERIZATION OF SUGARCANE TRANSCRIPTS RESPONSIVE TO WATER STRESS AIMING THE DEVELOPMENT OF TRANSGENIC DROUGHT-TOLERANT PLANTS

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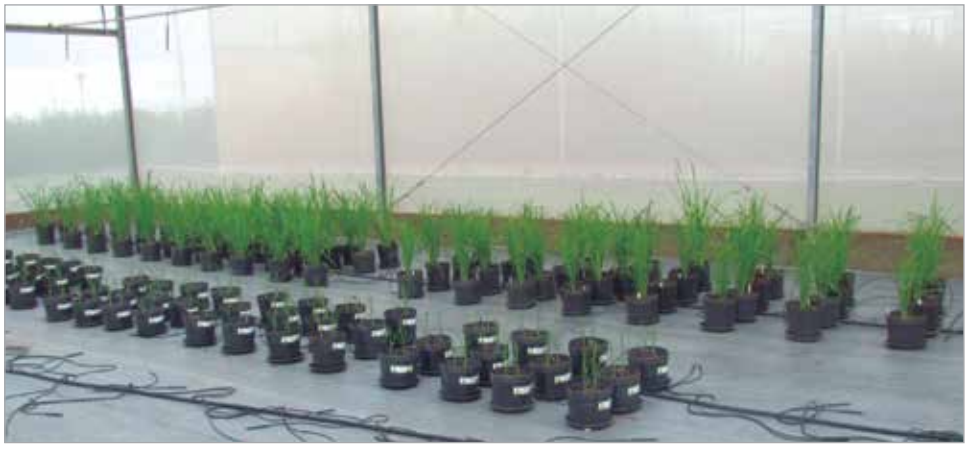

Figure 1. Rice transgenic plants at greenhouse overexpressing genes related to drought stress from sugarcane

Sugarcane (*Saccharum spp.*) is a promising source of renewable energy, and Brazil is the world leader of this crop. Significant yield losses have been observed in the last years, mostly because of drought conditions. For this reason, drought tolerance represents one of the most important targets in the development of transgenic sugarcane cultivars. However, despite of the limitations of the process in sugarcane transformation, mainly related to the low efficiency of the used methods, high dependence on genotype and gene silencing, the lack of well-characterized target genes to be used represents the main bottleneck in the development of transgenic sugarcane from a commercial point of view. Previous studies focused on global gene expression analysis (microarray and RNA-seq) using sugarcane genotypes contrasting for drought tolerance were conduced independently with experiments on field and at greenhouse, aiming to identify and characterize genes involved in drought tolerance. A set of differentially expressed genes identified and categorized as transcription factors (TFs) and miscellaneous classes were confirmed by qPCR. Two TF (bZIP_1 and MYB_2) and two miscellaneous were chosen for functional analysis through rice transformation (*Oryza sativa*), a grass model, evolutionarily close to sugarcane, but with well-established transformation procedures. Therefore, the full-length sequences of the coded sequences were accessed in SUCEST (Sugarcane Functional Genomics Database), Phytozome and NCBI database. The cloning was performed using the vector pGEM-T Easy (Promega) and E. coli DH10B lineage, and sequences subcloned into pDONR 211 Gateway vector. Overexpression vector construction, including Ubiquitin promoter (pEN-L4-UBIL-R1) and Hygromicin (*hpt*II) as selectable marker (pHb7m24GW), was used for *Agrobacterium tumefaciens*-mediated transformation of rice seeds. The rice transformation efficiency ranged from 50-60%, and the relative expression of transgenes was evaluated in transgenic plants for each target gene, using the $2^{-\Delta\Delta Ct}$ method. The transgene copy number was evaluated by a Taqman® assay based on *hpt*II gene. Analyses focusing on phenotyping and physiological evaluations were conduced in the second (T1) generation in four independent events for each target gene exhibiting only one copy number. Data analyses revealed that bZIP_1 has a role in drought tolerance in rice plants, whereas one gene categorized as miscellaneous resulted in an increase on biomass. Once confirmed the contribution of the genes here studied, these genes will be used in sugarcane genetic transformation aiming the development of cultivars with higher performance under restrictive water availability conditions.

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