Gene regulation and biotechnology of drought tolerance in sugarcane

Menossi, M.[1]; Barbosa, A.L.[2]; Barbosa, T.P.[3]; Begcy, K.[1]; Costa, M.D.-B,L.[4]; Gentile, A.[1]; Gonçalves, E.R.[3]; Hoshino, A.A.[1]; Lira, N.P.V.[5]; Mattos, R.[1]; Nishiyama-Jr., M.Y.[4]; Oliveira, E.T.[2]; Pestana-Calsa, M.C.[5]; Ramiro, D.A.[2]; Ribeiro, I.L.A.C.[5]; Veríssimo, V.[3]; Vieira, M.A.S.[6]; Vilela, R.D.[3]; Carneiro M.S.[6], Nogueira, R.J.M.C.[7]; Carrer, H.[2]; Calsa Jr., T.[5]; Endres, L.[3]; Souza, G.M.[4].

1 - Universidade Estadual de Campinas, Brazil; 2 - Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Brazil; 3 – Universidade Federal de Alagoas, Brazil; 4 – Universidade de São Paulo, Brazil; 5 – Universidade Federal de Pernambuco, Brazil; 6 – Universidade Federal de São Carlos, Brazil; 7 Universidade Federal Rural de Pernambuco.

Introduction

Drought is one of the main factors affecting sugarcane productivity. Sugarcane varieties differ in their ability to withstand drought while keeping sucrose accumulation. Considering that 70% of the water used by humankind is directed to irrigate crops, tolerance to water scarcity is also a key issue for agricultural sustainability. The understanding on the mechanisms underlying drought tolerance observed in some varieties will be valuable for the agronomical improvement of sugarcane. We have addressed the problem of drought tolerance in sugarcane using a multidisciplinary approach, integrating plant physiology, transcriptomics, microtranscriptomics, proteomics and biotechnology.

Results and conclusions

Sugarcane plants from three drought tolerant (RB92579, RB867515 and SP79-1011) and three drought sensitive varieties (RB72454, RB855536 e RB855113) were cultivated in the field in Alagoas. Plants were watered or not and leaf and first internode samples were taken three, seven and eleven months after planting (MAP). Several parameters were also evaluated, such as gas exchange, proline content, leaf area, internode diameter and productivity. RNA samples were extracted for gene expression profiling using Agilent arrays covering 14,522 sugarcane genes and also for the construction of miRNA libraries that were sequenced using Solexa. Protein samples were extracted for proteomic analysis. Selected genes were used to transform sugarcane and model plants aiming the production of plants with higher tolerance to drought.

Plant responses to drought included reduction of the leaf area, lower stomata conductance and lower leaf osmotic potential, as well as dynamic photoinhibition and induction of leaf proline content. No differences could be seen between the two groups (tolerant and sensitive) at three MAP. Photosynthesis was higher in drought tolerant plants at seven MAP and this difference was also noted at eleven MAP, although not at the same level as observed at seven MAP. Proline content had no correlation with drought tolerance in any of the three time points evaluated. As expected, plant productivity in the drought tolerant plants under drought conditions was higher

than drought sensitive plants.

The Agilent array was hybridized with probes derived from RNA samples extracted from leaves and the first internode from two drought tolerant sugarcane varieties (RB92579 and RB867515) and from one drought sensitive variety (RB92579). Hundreds of genes were modulated by drought in these two tissues in the three sugarcane varieties. Consequently, a wide array of protein functions was changed in response to drought. Interestingly, proteins involved in hormone biosynthesis, such as ABA and auxins, were found. We also found proteins related to folding, probably helping cells to overcome the stressful conditions that trigger protein malfunctioning. These classes of genes showed differential expression between the two drought tolerant varieties and the drought sensitive one.

To identify miRNA associated to drought tolerance, libraries were made from RB867515 and RB855536 leaves, seven MAP, under irrigation or not (drought treatment). Between 8 to 12 million valid reads were obtained by deep sequencing for each library. Conserved miRNA were identified based in their identity to the sequences in the miRBase, allowing two mismatches. The number of transcripts for each miRNA were normalized by the number of total reads in each library and used as an estimate of the miRNA abundance in each library. By comparing irrigated vs non-irrigated samples we were able to find 18 miRNA differentially expressed in the tolerant RB867515 and 9 in the sensitive RB855536. There were 9 miRNA that were found in both sugarcane varieties. Interestingly, most miRNA were repressed in the tolerant variety and the opposite was observed in the sensitive variety. *In silico* analysis identified the putative targets of these miRNA and indicated that a wide array of protein functions are affected, including transcriptional factors, phosphatases and helicases.

In spite of comprehensive transcriptomics, few information is available on sugarcane proteome associated to drought. Here, the approach is large-scale open definition of proteome by 2D-PAGE/MS in the sugarcane genotypes contrasting to drought tolerance. Initially, we have evaluated the proteome from plants grown in greenhouse and cultivated *in vitro*. Sugarcane plants were harvested after stress confirmed by water potential and relative water content. Leaves, stems and roots proteomes were defined by 2D-PAGE, resulting in distinct profiles between drought treatments, differing also in spots distribution along pH/MW ranges. Analyses allowed selection of differentially expressed peptides (DEP), exclusive or common spots. Those selected were identified by MS, and data suggested differential expression of proteins associated to drought response mechanisms, protein folding and photosystems composition. We observed several responses specific to some genotypes. Regarding the field-grown plants, 2-D allowed the identification of several spots that were modulated by drought. The identification of these proteins by MS is underway. Drought-DEPs identification will help functionally relevant biological data interpretation, and linkage to physiological profiles from the same samples. Proteins differentially expressed between these conditions may be used in selection and development of new sugarcane varieties with improved drought or salinity tolerance, attending the growing demand for renewable energy sources, as biofuels.

Aiming the functional evaluation of the genes and proteins identified in the

assays describe above, transgenic tobacco, Brachypodium and sugarcane plants overexpressing or silencing selected genes are being obtained. A new protocol for sugarcane transformation using sugarcane leaves was developed using biolistics. This approach certainly will allow us to verify the function of genes selected to increase drought tolerance in sugarcane. In a previous work (Rocha et al., 2007. BMC Genomics 8:71), using greenhouse grown plants we identified 93 genes that were modulated by drought stress. Two of these genes, encoding proteins with unknown function, were overexpressed in transgenic tobacco plants. These genes were named *Scdr1* and *Scdr2* (for Sugarcane drought-related) and they increased seed germination in media containing mannitol (simulating drought stress) and NaCl. Adult plants also showed higher tolerance to both mannitol and salt stress, presenting higher shoot biomass.

In summary, our data allowed an integrated picture of sugarcane responses when grown in the field under drought stress. Genomics and proteomics studies delivered an interesting list of genes that highlights the molecular mechanisms activated by sugarcane to cope with drought stress.

Supported by CNPq, FINEP, CAPES and FAPESP

Author publications

1. Vicentini, R. and Menossi, M. (2009). The predicted subcellular localisation of the sugarcane proteome. Functional Plant Biology, 36, 242-250
2. Vicentini, R., Felix, J.M., Dornelas, M.C., Menossi, M. (2009) Characterization of a sugarcane (*Saccharum* spp.) gene homolog to brassinosteroid insensitive1-associated receptor kinase 1 that is associated to sugar content. Plant Cell Reports, 28, 481-491
3. Gentile, A., Ditt, R.F., Dias, F.O., Silva, M.J., Dornelas M.C., Menossi, M. (2009). Characterization of *ScMat1*, a putative TFIIH subunit from sugarcane. Plant Cell Reports, 28, 663-672
4. Papini-Terzi FS, Rocha FR, Vêncio RZ, Felix JM, Branco DS, Waclawovsky AJ, Del Bem LE, Lembke CG, Costa MD, Nishiyama MY Jr, Vicentini R, Vincentz MG, Ulian EC, Menossi M, Souza GM (2009). Sugarcane genes associated with sucrose content. BMC Genomics, 10:Article Number: 120
5. Felix J.M., Papini-Terzi, F.S., Rocha, F.R., Vêncio, R.Z.N., Vicentini, R., Nishiyama-Jr, M.Y., Ulian, E.C., Souza, G.M., Menossi, M. (2009) Expression Profile of Signal Transduction Components in a Sugarcane Population Segregating for Sugar Content. Tropical Plant Biology, 2, 1935-9756.
6. Hotta, C.T., Lembke, C.G., Domingues, D.S., Ochoa, E.A., Cruz, G.M.Q., Melotto-Passarin, D.M., Marconi, T.G., Santos, M.O., Mollinari, M., Margarido, G.R.A., Crivellari, A.C., Santos, W.D., Souza, A.P., Hoshino, A.A., Carrer, H., Souza, A.P., Garcia, A.A.F., Buckeridge, M.S., Menossi, M., van Sluys, M., Souza, G.M. (2010). The

Biotechnology Roadmap for Sugarcane Improvement. Tropical Plant Biology, 3, 75-87.

7. Gentile, A., Da Cruz, P.L., Tavares, R.G., Krug, M.G., Dornelas, M.C., Menossi, M. (2010). Molecular characterization of ScTFIIAγ, encoding the putative TFIIA small subunit from sugarcane. Plant Cell Reports, 29, 857-864.