FUNCTIONAL´OMICS OF THE RATOON STUNTING DISEASE OF SUGARCANE

Luis Eduardo Aranha Camargo
Higher School of Agriculture "Luiz de Queiroz" / University of São Paulo (ESALQ/USP)
FAPESP Process | Term: Jun 2009 to Jun 2014 | Thematic Project

Figure 1. The disease causes significant reduction of biomass in susceptible cultivars of sugarcane. The "stunting" symptom reflects a shortening of the internodes of infected canes (left cane) compared to healthy ones (right cane)

The ratoon stunting disease (RSD) of sugarcane is caused by the fastidious xylem-limited gram-positive bacterium *Leifsonia xyli* subsp. *xyli* (Lxx). RSD is one of the most important diseases of sugarcane worldwide. Although control of the bacterium relies primarily on using healthy heat-treated stalks as planting material, this approach is not 100% effective and, given the perennial nature of sugarcane plants and the prevalent mechanical mode of transmission of the bacterium, the disease can reach epidemic levels during successive ratoon crops starting from a small amount of infected planting material. In Brazil, losses in biomass of sugarcane due to RSD are estimated to be around 3.3 million tons/yr or R$ 107 million/yr given the price of R$ 32/ton practiced in 2009.

The objectives of our study are a) to establish a time course of colonization of sugarcane by Lxx using the quantitative real time (q)PCR approach in order to identify time points that encompass the onset of the plant reaction to infection; b) to identify sugarcane genes and proteins differentially expressed in a resistant and a susceptible cultivar infected or not with Lxx based on microarray technology at the time points previously defined; c) to characterize the biological effects on sugarcane plantlets of a presumed toxin-like compound secreted by Lxx and study its effects on gene expression in plants cultivated *in vitro*. In addition, genes thought to be involved in the production of this toxin will be characterized by heterologous expression, purification and analysis by mass-spectrometry.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The lack of information regarding the time course of colonization of sugarcane by Lxx prompted us to focus on the establishment of protocols for the inoculation and quantification of Lxx in plant tissue by qPCR. With this information, we were able to define time points for gene and protein expression analyses. We then used 2D-DIGE to compare changes in protein profiles of plants colonized with low and high titers of Lxx (*Figure 2*). Plantlets of the variety SP80-3280 naturally infected with Lxx were either mock inoculated (low titer treatment) or inoculated with a suspension of Lxx CTCB07 (high titer treatment). Proteins were extracted 30 and 60 days after inoculation (DAI) from leaf whorls and profiles were compared within treatments over time. Quantitative PCR of plant tissue at 30 and 60 DAI indicated that the bacterial population remained low in the first treatment, whereas in the second it increased tenfold. Thirteen and 68 differentially expressed proteins were uniquely detected in the low and high titer treatments, respectively. Protein identification by MS indicated that, in the first case, they were mainly categorized as involved in stress responses. In the second case, however, proteins were functionally more diverse. Noteworthy were markedly downregulated proteins involved in plant growth such as calreticulin and cyclin, and upregulated proteins involved in hormone reception (ABA and JA) and in responses to osmotic stress. These results are consistent with the main symptom of the disease and indicate that temporal changes in protein expression associated with increased bacterial titers could result from altered hormonal balance (*Figure 2*).

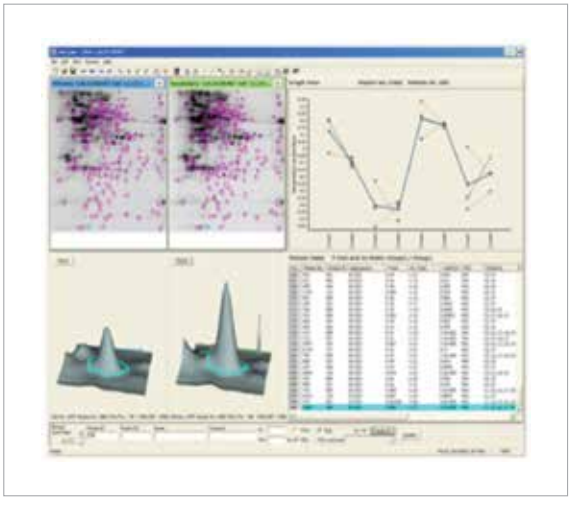

Figure 2. 2D-DIGE profile and quantification analysis using DeCyder software

MAIN PUBLICATIONS

Monteiro-Vitorello CB et al. The genome sequence of the Gram-positive sugarcane pathogen *Leifsonia xyli* subsp. *xyli*. 2004. *Molecular Plant-Microbe Interactions*. **17**: 827-836.

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Luis Eduardo Aranha Camargo

Escola Superior de Agricultura Luiz de Queiroz
Universidade de São Paulo (USP)
Departamento de Fitopatologia
Av. Pádua Dias, 11 – Caixa Postal 9
CEP 13418-900 – Piracicaba, SP – Brasil

+55-19-3429-4124 r. 212
leacamar@esalq.usp.br