FUNCTIONAL ´OMICS OF THE RATOON STUNTING DISEASE OF SUGARCANE

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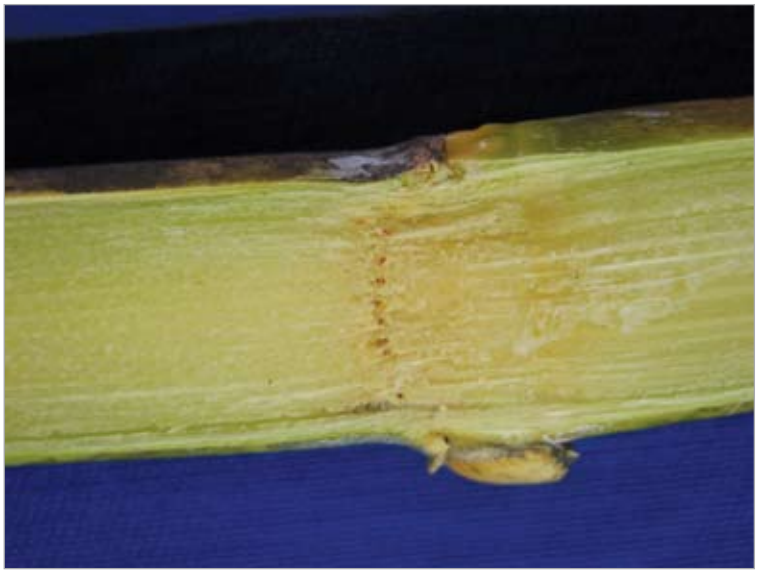

Figure 1. Internal symptoms caused by Leifsonia, characterized by small redish dots below the plant internodes. Infection by this bacterium is characterized by the absence of conspicuous external symptoms

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

Analyses of gene or protein expression provide a snapshot of an ongoing biological process. Thus, it is crucial to determine when the snapshots will be taken so as to get meaningful answers to the questions being asked. 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 in this first semester. With this information, we can define time points of subsequent gene and protein expression analyses. Primers for a Lxx-specific ORF were designed based on its available genome sequence and successfully used in qPCR to quantify Lxx in leaves of young plants. Plants grown *in vitro* (susceptible and resistant varieties) were transplanted to the greenhouse and inoculated after 60 days by cutting them just above the apical meristem and placing a volume of inoculum on the cut surface. Bacterial populations were estimated in leaf DNA extracts 10, 20, 40 and 80 days after inoculation. For optimal quantification of Lxx by qPCR, we found it necessary to extract the plant DNA using a combination of the surfactants SDS and CTAB with proteinase K and lysozyme. The results indicated a rapid in planta growth that sharply contrasts with the slow growing behavior of Lxx in artificial medium. This suggested that a sizeable colonization of sugarcane tissues occurs well before the manifestation of external symptoms, which happen at least 9 months after inoculation. Thus, changes in gene and protein expression in infected sugarcane may occur in a much shorter time span than that expected previously. In addition, we detected differences in bacterial titers between inoculated resistant and susceptible varieties. Further studies should be pursued to determine if this rapid inoculation and quantification method reflects the level of resistance displayed by sugarcane varieties under field conditions. If so, a side result of this study with practical implications would be the use of this technique to select resistant genotypes.

Figure 2. 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)

MAIN PUBLICATIONS

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