Development of Protein and Transcript Databases Expressed During *Panicum Maximum* X *Bipolaris Maydis* Interaction, Using Data Generated From High-Throughput Sequencing Methods

K. Guimarães X. Meireles[1], L. Jank[2], C. Fernandes[3], D. Braga[4]

[1]Plant Biotechnology Laboratory, Researcher of Embrapa Beef Cattle, Campo Grande-MS, Brazil;
[2]Genetic Breeding of Forage, Researcher of Embrapa Beef Cattle, Campo Grande-MS, Brazil,
[3] Phytopathology Laboratory, Researcher of Embrapa Beef Cattle, Campo Grande-MS, Brazil;
[4]Plant Biotechnology Laboratory, Undergraduate Scholarship from Embrapa Beef Cattle.

Panicum maximum is a C4 grass of African origin, widely used in pastures in Brazil, where it is grown on approximately 15 million hectares. Cultivars Mombaça, Massai and Tanzânia of *P. maximum*, released by the Brazilian Agricultural Research Corporation – Embrapa, are extensively adopted in the beef and milk production systems in the country, due to their wide adaptation to several types of climates and soils, high productivity and excellent agronomic characteristics for use as forage. The Brazilian Market on 2012 for certified seeds of these three cultivars was around US$ 85 million. Over the past decade, *P. maximum* pastures have been threatened by the increasing occurrence of leaf spot, a disease very well known in the country due to the severity of damage to the corn crop in the 1970s and 1980s. The leaf spot in *P. maximum*, caused by the fungus *Bipolaris maydis*, is characterized by lesions on the leaves of the grass of varying sizes and shapes, usually around 0.3-1.0 cm. In more severe attacks, these lesions coalesce into larger necrotic dark areas, and practically all aerial parts are affected. Due to the high susceptibility to *B. maydis* observed in cultivar Tanzania, which resulted in significant decrease in area of cultivation, as well as frequent moderate damage to pastures of cultivars Mombaça and Massai, Embrapa's *P. maximum* breeding program included in its routine, the characterization for resistance to *B. maydis* of its germplasm and promising genotypes for forage use in various stages of evaluation in the field. With the ongoing reduction in cost and resources required to provide molecular tools to underpin molecular breeding strategies, the breeding program recently incorporated transcriptomics and proteomics approaches aimed at understanding the molecular interaction between *P. maximum* and *B. maydis*. Total RNA extracts were obtained in triplicate from plants of a resistant genotype and of a susceptible one to *B. maydis*, maintained in interaction with the fungus for 24h, 48h and 72h, as well as their respective controls (plants with no contact with the pathogen). Twenty-four cDNA libraries were prepared from RNA samples and sequenced on Illumina platform HiSeq2000, resulting in billions of reads of 100bp. For the proteomic analysis, total proteins were extracted from 2.0 g of leaf tissue from all the treatments mentioned above. The samples were desalted and digested with trypsin, followed by peptide analysis by mass spectrometry, which is underway. In this innovative methodology, a sample of peptides is pre-fractionated by three chromatographic columns (nanoACQUITY system), followed by mass spectrometry analysis model Synapt G2, which allows absolute quantification of proteins and characterization of thousands of molecules in a single sample, including those of low abundance. The development of a differentially expressed peptide database will assist in the understanding of mechanisms of cellular response of the forage to stress caused by *B. maydis*. Genetic engineering of plants may in the future benefit from this knowledge through the identification of gene products that can confer resistance to the pathogen. In this paper, we present the results of bioinformatics analysis and assembly of de novo transcriptome of *P. maximum* x *B. maydis*. As this specie is poorly represented in genome sequence databases, the resulting contigs are being compared to the gene complement from the related model grass species. Thus, this study will provide, in an unprecedented manner, the first catalog of transcripts of *P. maximum*. Bioinformatics analyzes include detection of differential expression of transcripts in each time of forage x pathogen interaction, aiming at the identification of microsatellite markers for resistance to *B. maydis*, as well as the selection of candidate genes for this trait. This molecular marker resource will enable future molecular breeding efforts in *Panicum maximum*.

Corresponding author: Karem Guimarães Xavier Meireles
karem.meireles@embrapa.br