Analysis of expression of xylanases encoded in the genome of rust coffee fungus during different stages of infection

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Abstract

Coffee leaf rust is a major disease caused by the fungus Hemileia vastarix that affects many coffee producers around the world. Since H. vastatrix is a biotrophic fungus, its growth and reproduction are totally dependent on the cells of the living host, and because of that, they infect the tissue without causing necrosis. Also, it is known that some fungi, during plant interaction, can express genes involved in the formation of infectious structures as well as synthesize enzymes responsible for the degradation of the host cell wall. Many of them produce enzymatic cocktails capable to degrade cell wall components, which are basically cellulose, hemicelluloses and lignin. The most abundant group of hemicellulases are xylans, which has aroused industrial interest for many applications, such as biobleaching in the pulp and paper industry and as prebiotics in animal nutrition. However, more studies are needed to evaluate interactions between fungi-plant and other factors that can activate fungus pathogenicity. Also, it is desirable to identify active xylanases of commercial interest. Because of that we propose on this work to evaluate if genes that encode xylanases are being expressed during H vastatrix infection. First, the fungal protein was predicted by Augustus ab initio prediction. The program was set for the fungus Puccinia graminis. The functional annotation of the predicted genome was performed by dbcan2 (release 8.0) selecting all CAZymes from the genome. Among the 345 CAZymes found, 162 belongs to the Glycoside Hydrolases (GH) and only 3 are xylanases (GH10). After that, was performed the analysis of RNA-seq data of C. arabica cv. caturra vermelho CIFC 19/1 (Bioproject: PRJNA353185) to 0, 12, 24, 96 hai. Read quality was assessed with FastQC software version 0.11.5 and trimmed with Trimmomatic software version 0.36. Next the "Tuxedo" pipeline was executed using Hemileia vastatrix HvCat (PZQQ00000000.1) as the reference genome – the same used to gene prediction in Augustus. The preliminary results indicate that the 3 xylanases present different expression profile, but are being most expressed in the early stages of the infection: 12 and 24 hai, which corresponds to the phases when the fungus is penetrating the plant. For the next steps of this work we aim to perform gene expression analysis of the 3 xylanases using real-time quantitative PCR and execute activity assays with the xylanases.

Funding:

Link to Video: