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Gene expression analysis in maize ears and silks after *Fusarium verticillioides* infection

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The genus *Fusarium* includes numerous plant pathogens that cause destructive diseases on some of the world's most agriculturally important plant species, including maize. In particular *F. verticillioides* is one of the most economically important *Fusarium* species that causes root, stalk and ear rots, blights and wilts, and can contaminate kernels with a family of closely related mycotoxins known as fumonisins.

Plant defenses consist of physical barriers such as cell wall and its modifications as well as chemical desense mechanisms that are induced in response to external stimuli. In addition plant defense involves expression of pathogenesis-related (PR) proteins (Kitajima and Sato, 1999 J Biochem 125:1-8). PR proteins are grouped into 17 indipendent families and antimicrobial properties have been described for some of them. The PR-2 proteins display β-1,3-glucanase activity, whereas the PR-3 proteins (as well as PR-4, PR-8 and PR-11 proteins) show endochitinase activity (Campo et al., 2004 Proteomics 4:383-396).

In maize few detailed studies are presently available on its response to *F. verticillioides* infection and although differences in susceptibility to this pathogen attack have been described, resistant mazie varieties have not yet been developed (Bluhm and Woloshuk, 2005 Mol. Plant-Microbe Interactions 18:1333-1339). Objective of the research was the identification of genes expressed in kernels and silks of maize tolerant and susceptible lines, during *F. verticillioides* infection, using the microarray technology.

The tolerant line CO441 and the susceptible line CO354 were used. Their ears were infected with a fumonisin-producing strain of *F. verticillioides*, with the pin-bar technique and harvested 48 hours after infection and also from uninfected ears. RNAs extracted, reverse transcribed and labelled with fluorophor dyes, were hybridized on the array slides. Venn Diagrams showed that only 60 genes were differentially expressed in CO441 e CO354 48h after infection. Most of genic sequences were peculiar to both the lines. Similar functional categories of genes were involved in the response to

infection in both tolerant and susceptible lines, such as: defense response proteins, oxidative burst-associated enzymes, enzymes involved in sugar metabolism and proteins involved in aminoacid synthesis, folding and stabilization. The most significant differentially expressed genes were validated in RealTime-PCR.

The main PR genes identified in the array experiments were also tested in silks, infected and harvested 12, 24, 48 and 72h after infection, and from uninfected samples using RealTime-PCR.

To monitorate the presence and the activity of *F.verticillioides* through absolute quantification in seeds and silks, the costitutive gene for the β-tubulin and the target gene *FUM21*, a transcriptional regulator of fumonisins metabolic pathway, of *Fusarium verticillioides* were also analyzed. The results showed a higher copy number for both the genes in the susceptible line CO354.