Gene expression analysis of wheat near isogenic lines for *Fhb1* and *Qfhs.ifa-5A* after *Fusarium graminearum* inoculation

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INTRODUCTION

Fusarium head blight (FHB) is one of the most destructive diseases of wheat worldwide. In this research work we aim to identify expressed genes involved in FHB resistance of wheat, in particular to identify genes/alleles associated with the two major resistance QTL Fhb1 and Qfhs.ifa-5A. The tool used for identification of target genes are Affymetrix microarrays.

MATERIALS and METHODS

The highly resistant line CM82036 and four BC5F2 near isogenic lines (NILs) for Fhb1 and Qfhs.ifa-5A were used for the microarray experiments. At anthesis the lines were challenged by F. graminearum or water. Inoculated wheat florets were dissected and the transcriptome of the wheat lemma, palea and the subtending section of the rachis (Figure 1) was analyzed at three time points after inoculation (8, 24, 72 hai, hours after inoculation) in three biological replications.

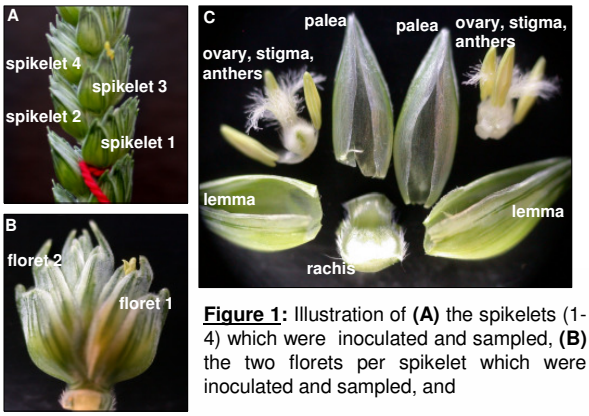

Figure 1: Illustration of **(A)** the spikelets (1-4) which were inoculated and sampled, **(B)** the two florets per spikelet which were inoculated and sampled, and **(C)** the floral tissues used for RNA preparation, separated in the reproductive part (ovary, stigma, anthers) and the lemma, palea and the subtending section of the rachis.

Total RNA was extracted and sent to the University of Minnesota. All labeling, hybridization and data acquisition were performed at the Microarray Facility of the University of Minnesota.

The statistical analysis of the microarray data is still in progress using the R statistical analysis environment (http://www.r-project.org) and packages of the Bioconductor suite (http://www.bioconductor.org). Probe sequence-specific background correction was performed using the Bioconductor 'gcrma' package (Wu et al., 2004), interchip normalization could be achieved using 'vsn' package (Huber et al., 2002) and robust summaries of probe-set signals were obtained through the fitPLM function of the package 'affyPLM' (Bolstad, 2004). The normalized data were then fitted gene by gene with a linear model, using the lmFit function (Smyth et al. 2007) of the Bioconductor package 'limma'. To identify statistically significant differentially expressed probe sets q values after correction for multiple testing controlling the false discovery rate were calculated (Benjamini and Yekutieli, 2001).

PRELIMINARY RESULTS and DISCUSSION

Differences in gene expression related to the treatment, genotype and time point were detected. Figure 2 summarises the numbers of differentially expressed transcripts after *Fusarium* inoculation at 8, 24 and 72 hai for CM82036, NIL1 (*Fhb1* and *Qfhs.ifa-5A*), NIL2 (*Fhb1*), NIL3 (*Qfhs.ifa-5A)* and NIL4 (susceptible alleles at *Fhb1* and *Qfhs.ifa-5A*).

In total, 16 (8 hai), 162 (24 hai) and 1348 (72 hai) transcripts showed pathogen-responsive expression patterns (q ≤ 0.05, twofold difference).

In addition, we identified 29 constitutively expressed transcripts specific for the *Fhb1* QTL region. 11 of these transcripts exhibited an increase whereas 18 exhibited a decrease in the lines carrying *Fhb1* compared with the lines carrying the susceptible allele at *Fhb1*.

For *Qfhs.ifa-5A* we revealed 57 transcripts with differential expression between lines possessing either the resistant or the susceptible alleles independently from the treatment. 33 transcripts were up-regulated whereas 24 were down-regulated in lines with *Qfhs.ifa-5A* compared to the susceptible allele.

In the most interesting group of differentially expressed transcripts QTL-specific and pathogen-responsive genes were identified, with mainly up-regulation in the NILs lacking *Qfhs.ifa-5A*.

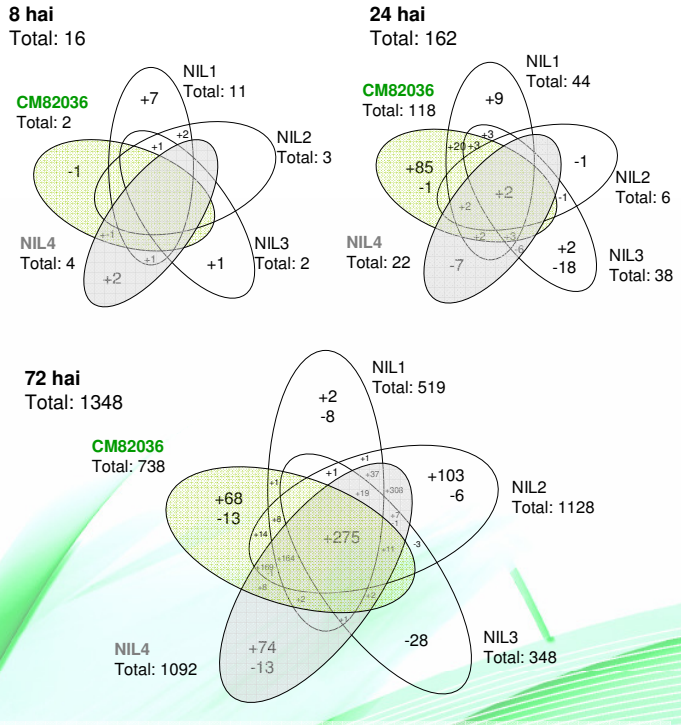

Figure 2: The numbers of pathogen-responsive transcripts at 8, 24 and 72 hai for CM82036, NIL1 (*Fhb1* and *Qfhs.ifa-5A*), NIL2 (*Fhb1*), NIL3 (*Qfhs.ifa-5A)* and NIL4 (susceptible alleles at *Fhb1* and *Qfhs.ifa-5A*). (+) up-regulation and (-) down-regulation after Fusarium inoculation, in reference to mock inoculation. FDR cutoff of q < 5%, after correction for multiple testing and fold-change ≥2.

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