A database of RNA profiles comparing susceptible and resistant wheat infected with *Fusarium graminearum*

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INTRODUCTION

Diseases caused by the fungus *Fusarium graminearum* Schwabe (*Fg*; teleomorph *Gibberella zeae (*Schw.) Petch) constitute one of the major problems in cereal crops grown in temperate climates worldwide. *Fg* can cause severe epidemics of fusarium head blight (FHB) in wheat and barley, and gibberella ear rot in maize, as well as seedling blights and stalk rot (Goswami and Kistler, 2004). In addition to causing significant yield losses, *Fg* produces mycotoxins, including deoxynivalenol and zearalenone, which affect grain quality and present health hazards for both humans and animals (CAST 2003). There are also concerns that deoxynivalenol, which is a strong protein synthesis inhibitor, might affect the plant's ability to respond to *Fg* infection.

So far, conventional breeding approaches have produced limited success in improving the resistance of cereal crops,to *Fg*. However recent progresses in mapping have led to the identification of many quantitative trait loci associated with FHB resistance in wheat and barley, with promises to speed up the breeding process (Somers et al, 2005).

To complement the mapping approach and better understand the molecular mechanisms of the wheat response to *Fg* in susceptible and resistant wheat varieties, a genomics approach has been used. Microarray hybridization experiments have been conducted using the Affymetrix GeneChip Wheat Genome array (representing ca. 54,500 expressed sequences), comparing mock-inoculated and *Fusarium*-inoculated wheat varieties. All profiles have been compiled into a database using the softwares Acuity and SQL Server.

We are interested into the following questions:

A) What are the key differences between resistant and susceptible responses at the molecular level?

B) What are the commonalities and differences between type I and type II responses, and between all resistant sources?

C) Are there differences at the molecular level between response to point and spray inoculation?

D) Can we associate specific parts of the RNA profile response to specific QTLs? (not quite sure what you mean)

E) What is the impact of deoxynivalenol, an inhibitor of protein synthesis, on the plant response?

This paper will describe the material used for the microarray analyses, the steps used to build the database and present preliminary interpretation of the data.

WHEAT MATERIAL ANALYSED BY MICROARRAY

Our experiments have focused on the early phase of infection by *Fg* on wheat heads. Depending on the type of FHB resistance present in the material analysed, either spray or point inoculation of the heads was used. Wheat lines and varieties carrying type II resistance (resistance to spread of initial infection) were inoculated using point inoculation (1000 *Fg* spores/10 µl) of two florets per spikelet, and all developed spikelets of 5 to 8 heads at mid-anthesis were inoculated for each sampling point. Inoculated spikelets were then harvested at 1, 2 and 4 days after inoculation. Heads of wheat varieties carrying type I resistance (resistance to initial infection) were spray inoculated (100,000 *Fg* spores/ml) to saturation. Whole heads or spikelets were harvested at 1, 3 and 6 days or only at 4 days, depending on the experiment. For each experiment, 2 or 3 biological replicates were performed and analysed. In addition to performing microarray hybridization analysis, the samples were tested for fungal load (using quantitative PCR of ß-tubulin to quantify fungal DNA) and for the mycotoxin deoxynivalenol (using a quantitative ELISA assay). These additional analyses allowed making a direct correlation between the plant response and the fungal activity in the samples.

We have obtained the RNA profiles for six groups of wheat plants:

1) Spring wheat varieties Roblin (very susceptible), Wuhan 1 and Nuy Bay (respectively a type II resistant from China and an unclassified resistant type form Japan);

2) Near isogenic lines, derived from the cross Wuhan 1 x Nuy Bay, that segregate for the QTLs 2DL, 3BS and 5AS which are associated with FHB resistance

3) Spring wheat Chinese Spring (moderately susceptible) and the addition lines 7E and 7ES. (both type II resistant, containing the chromosome 7 from *Thinopyrum elongatum* into Chinese Spring background);

4) Winter wheat Augusta (susceptible) and FHB148 (type I resistant, derived from Frontana, a Brazilian source of resistance);

5) Winter wheat Dream (unclassified resistant type from Germany)

6) Susceptible spring wheat variety Roblin, inoculated with either a wild-type *Fg* strain or a TRI5- derivative of it which does not produce deoxynivalenol nor any intermediates.

ASSEMBLY OF THE MICROARRAY PROFILE DATABASE

Our first challenge in performing a large scale data analysis was to combine individual datasets into one database without creating artifacts when comparing across datasets. Attempts to combine and normalize all six separate microarray experiments together into a megaset indicated that too many artifacts/bias were introduced during the process. We implemented a solution into two main steps which is illustrated in Figure 1:

Figure 1. Assembling a microarray profile DB

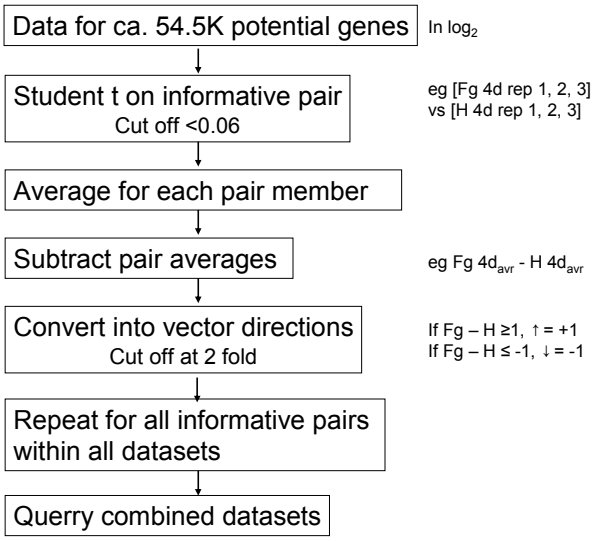

Table 1. Example of vector table with a subset of genes

AcuityID	possible gene function	Roblin	Wuhan 1	Nuy Bay	-2DL	+2DL	CS	7ES	Roblin/ FG-DON	Roblin/ FG+DON
TaAffx.548.1.A1_s_at	60S ribosomal protein L18a	0	0	0	0	-1	0	0	0	0
Ta.8399.1.S1_at	cytochrome P450	1	0	0	1	1	1	1	1	1
Ta.25832.1.A1_at	cytochrome P450	0	0	0	0	0	0	0	0	-1
TaAffx.112043.1.S1_at	eukaryotic translation initiation factor eIF-5A-2	0	0	0	-1	0	0	0	0	0
Ta.26928.1.S1_a_at	fructan exohydrolase	-1	0	0	-1	-1	0	0	0	-1
Ta.10966.1.S1_at	geranylgeranyl transferase	0	0	0	0	-1	0	-1	0	0
Ta.12469.1.A1_at	hydroxyanthranilate hydroxycinnamoyltransferase	0	0	0	0	0	-1	-1	0	0
Ta.30534.1.S1_at	senescence-associated protein	-1	0	0	-1	-1	0	-1	0	0
TaAffx.31754.1.S1_at	serine/threonine kinase receptor precursor-like protein	1	0	1	1	1	1	1	1	1
Ta.7079.1.A1_at	similar to speckle-type POZ protein	-1	0	0	-1	-1	0	0	0	-1
Ta.10097.1.S1_at	wpk4 protein kinase	0	0	0	-1	0	0	0	0	0
Ta.1290.2.S1_at	unknown	0	0	0	-1	-1	0	0	-1	-1
Ta.19960.1.A1_at	unknown	0	1	1	1	1	1	1	0	1
Ta.22541.1.A1_x_at	unknown	0	0	0	-1	-1	0	0	0	-1
Ta.9102.1.S1_at	unknown	-1	0	0	-1	-1	0	0	0	0
TaAffx.105286.1.S1_at	unknown	1	0	0	1	1	0	0	0	1
TaAffx.106064.1.S1_at	unknown	1	0	1	1	1	0	0	1	1
TaAffx.92629.1.S1_at	unknown	-1	0	0	0	-1	0	0	0	-1

A) Perform a Student t-test on comparison pairs to eliminate genes with high variation between biological replicates.

B) Create a vector table using direction of change rather than absolute data (vectors +1, 0 or -1). Table 1 is an example with a subset of genes and datasets

The vector table allows us to quickly look at the behavior of groups of genes across many datasets. The observations done so far indicate that:

1) The number of genes upregulated in Fg-infected samples is proportional to the level of infection in the samples as measured by Fg DNA amounts.

2) The level of infection in spray inoculated samples is much lower than those from point inoculation, making their RNA profiles only marginally different from those of the water-treated samples

3) Genes upregulated in resistant varieties are a subset of genes upregulated in susceptible ones

4) Close to 50% of the genes with an interesting profile of expression have an unknown function

5) Lists of candidate genes correlating with resistance will be generated, however additional validation and testing will be required to determine which genes are truly contributing to resistance.

REFERENCES

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