

IDENTIFICATION OF ESTs THAT ARE POSSIBLY RELATED TO THE FHB-RESISTANCE OF WHEAT (*TRITICUM AESTIVUM L.*) CULTIVAR SUMAI 3

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The epidemics of FHB (Fusarium head blight) can cause a great loss of wheat (*Triticum aestivum L.*) production. The purpose of this research is to investigate the genetic basis of FHB-resistance at the molecular level and pave the way for marker-assistant breeding of FHB-resistant wheat. Spring wheat cultivars Sumai 3 (FHB-resistant) and Wheaton (FHB-susceptible) were studied with RNA differential display and single-floret inoculation with a FHB isolate was used to initiate FHB. Several expressed sequence tags (ESTs) were revealed only in FHB-inoculated Sumai 3. We are characterizing these ESTs.

Keywords

Fusarium head blight, wheat, differential display

INTRODUCTION

Fusarium head blight (FHB, also known as "scab"), mainly caused by fungus *Fusarium graminearum*, is a devastating disease affecting all classes of wheat and other small grains. FHB can occur to wheat from heading to harvest and cause the failure of kernel development, scabby "tombstone" kernels and kernels contaminated with vomitoxin. The epidemic of FHB in recent years have resulted in a great loss in wheat production and the related food industries (McMullen 1997). About \$2,606 million dollars of loss in American agriculture and food industries between 1991 and 1996 was estimated by Johnson et al (1997).

Breeding resistant wheat cultivars is an efficient way to fight FHB. Several sources of resistance have been identified. Different kinds of resistance were revealed (Schroeder et al. 1967, Meidaner 1997). However, the multiple gene nature of wheat resistance to FHB (Bai et al. 1989; Guo 1989; Singh et al. 1995; Ginkel et al. 1996) make the conventional breeding method time- and labor-consuming and low in efficiency. Also, the application of marker assistant breeding and gene transformation were limited due to the lack of basic information about FHB pathogenesis at the molecular level. Muehlbauer et al. (1999) investigated the expression of several common defense-related genes in

wheat spikes 48 hours after *F. graminearum* inoculation. No significant difference was found between resistant and susceptible wheat cultivars, although the expression of those common defense-related genes were induced strongly.

Establishment of the infection results from complex interaction between plant and fungus, involving the expression of resistant genes of the plant and virulent genes of the fungus. In both organisms altered gene expression occurs from the onset of the attempted fungal invasion onwards. These genes, which are induced specifically by each other (the host resistant genes by pathogen; the pathogen virulent genes by host), could be identified with differential display of mRNAs between the resistant and susceptible cultivars.

The PCR based mRNA differential display technique (DDRT-PCR) developed by Liang and Pardee (1992) is a sensitive and quick method for the analysis of differential gene expression. The principle of DDRT-PCR is to use a set of oligonucleotide primers, one being anchored to the polyadenylate tail of a subset of mRNAs, the other being short and arbitrary in sequence so that it anneals at different positions relative to the first primer. The mRNA subpopulations defined by these primer pairs were amplified after reverse transcription and resolved on a DNA sequencing gel. This technique has been successfully used for the gene expression analysis in a plant-fungus interaction in tomato (Benito et al. 1996).

The purpose of this research is to identify the specific FHB-resistant genes of wheat and the virulent genes of *F. graminearum*, to provide molecular markers for breeding resistant cultivar and to enrich our understanding of molecular mechanism of FHB pathogenesis.

MATERIALS AND METHODS

Spring wheat cultivars Sumai 3 (FHB-resistant) and Wheaton (FHB susceptible) were used in this research. Plants were grown in a greenhouse on SDSU's Brookings campus. Single-floret inoculation was conducted at the beginning of anthesis to initialize FHB development. 20 μ l of conidiospore suspension (100,000 spores/ml) of *F. graminearum* isolate Fg4 or distilled water (used as control) was placed between the lemma and palea of the first flowering floret in the middle of a wheat spike with a pipette. The inoculated plants were immediately moved into humidity chamber until the floret was harvested (for the treatments less than 24 hours) or for 24 hours (for treatments more than 24 hours). Caution was taken to prevent inoculum from contaminating other florets. The inoculated and adjacent 4 spikelets were collected in 0, 1, 16, 32 and 64 hours after FHB and water inoculation. Samples are immediately frozen in liquid nitrogen and stored at -80°C until use.

High-quality, total RNA was extracted from the samples with Tri Reagent (Molecular Research Center, Inc. Cincinnati, OH, USA). Differential display of mRNA was conducted with RNAimage™ Kit (GenHunter, Nashville, TN USA). The protocol provided with the kit was followed with necessary modification. Generally, the subsets of mRNAs were defined by the M nucleotide of the dT_nM (M is the A, C or G degeneracy) primer anchored to the polyA tail of mRNAs and reversely transcribed. The subsets of cDNAs were then amplified

with the combination of the corresponding dT₁₁M primer and an arbitrary 13mer primer, which is annealed at a different position relative to the dT₁₁M primer. A total of 50 different primer combinations were tested for displaying differentially expressed mRNAs in the sampled wheat spikelets. The produced ESTs were resolved on 6% denatured acrylmide DNA sequencing gels and visualized with silver staining (Bassam 1991).

RESULTS AND DISCUSSION

The response of Sumai 3 and Wheaton after inoculation

Three independent experiments showed that the response of Sumai 3 and Wheaton to FHB isolate Fg4 is very consistent. When the samples were collected 32 hours after inoculation, the inoculated single florets of both Wheaton and Sumai3 have got light FHB symptoms (brownish spots at the glumes); But no FHB symptom was observed on the florets next to the inoculated single florets. However, 8 days later after inoculation, the half FHB-treated Wheaton spikes were bleached and dry and no kernel development was observed. In the case of Sumai 3, the FHB symptoms spread only to the rachis next to the inoculated single florets (Fig. 1) and the kernels developed well. These results indicate that the FHB inducing procedure is very suitable to demonstrate the type II resistance of Sumai 3.

Differential Display

Using 50 primer combinations, a total of 30,210 ESTs were recognized in the spike samples, with an average of 40.8 (17-60) per primer combination for



Figure 1. The responses of Sumai 3 and Wheaton to *F. Graminearum* 10 days after inoculation.

each sample. Of these ESTs, 800 are polymorphic among the treatments, an average of 3.2 (0-14) per primer combination. Our data revealed several types of gene expression patterns (Fig. 2). For example, Some genes were expressed only in the spikes of either FHB inoculated Wheaton (Fig. 3a), FHB-inoculated

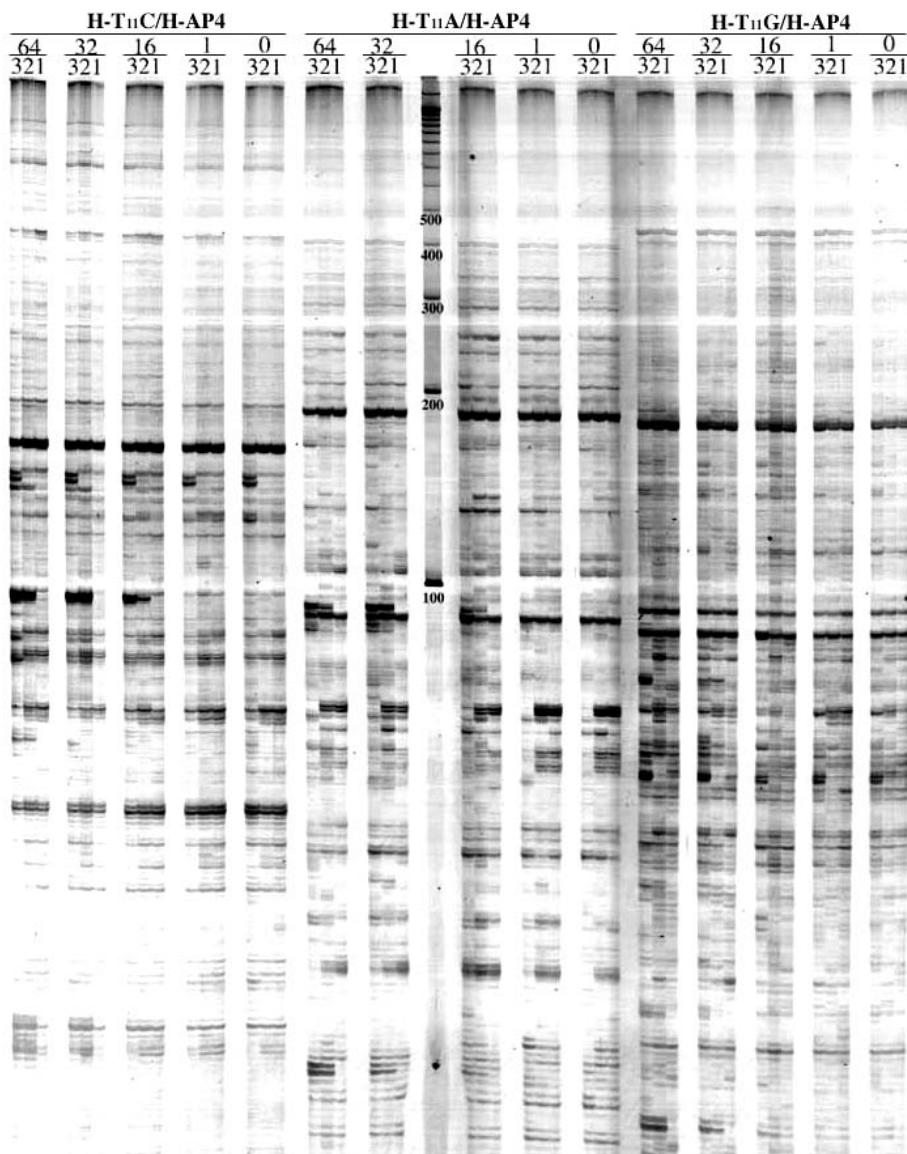


Figure 2. An example of mRNA differential displays of water-inoculated (1) spikes of Sumai 3 and the FHB-inoculated spikes of Sumai3 (2) and Wheaton (3). Samples are collected in 0, 1, 16, 32, 64 hours after inoculation. Primer combinations used are AP4+H-T₁₁A, AP4+H-T₁₁C and AP4+H-T₁₁G.

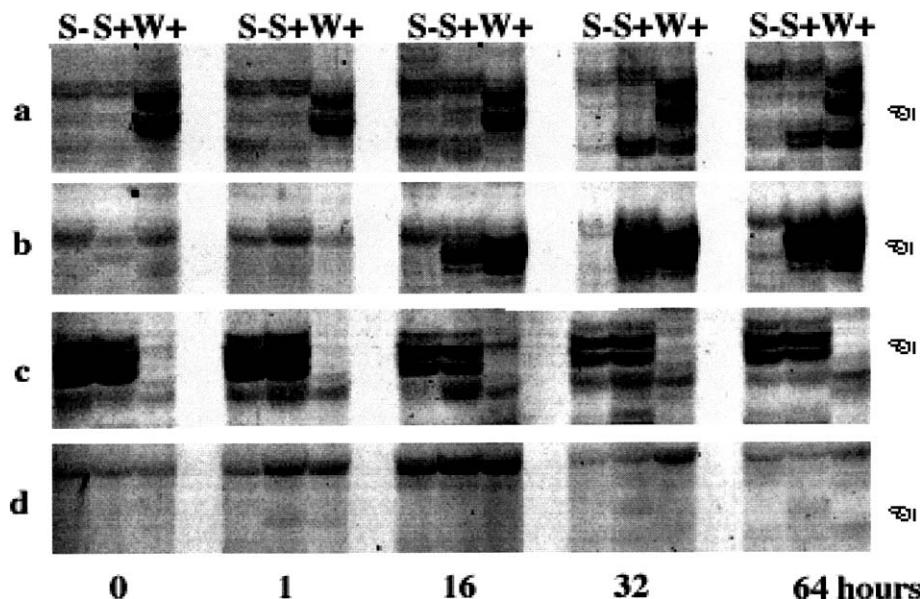


Figure 3. Examples of differentially expressed genes in FHB-inoculated (+) or water-inoculated (-) spikes of a FHB-resistant wheat cultivar Sumai 3 (S) and a FHB-susceptible cultivar Wheaton (W) revealed by mRNA differential display. Samples are collected in 0, 1, 16, 32, 64 hours after inoculation. a: two ESTs expressed only in Wheaton; b: one EST expressed 16 hours after FHB-inoculated but is absent from the water-inoculated control; c: three ESTs expressed only in Sumai 3 and one EST showed increased expression in FHB inoculated Sumai 3; and d: one EST showed increased expression in FHB-inoculated Sumai3.

Wheaton and Sumai 3 (Fig. 3a, b), or the water- and FHB-inoculated Sumai 3 (Fig. 3c). Genes that expressed only in FHB-inoculated spikes of Sumai 3 (Fig. 3d) or change their expression level at certain time after FHB-inoculation (Fig. 3a, b, c, d) were also observed. Those ESTs that appeared in both FHB-inoculated Sumai3 and Wheaton spikes may be accounted for general resistance. It is also possible that they were resulted from the expression of Fusarium virulent genes. Those ESTs that expressed only in the FHB-inoculated Sumai 3 spikes are the most promising and are possible ones that are specifically related to FHB resistance in Sumai 3. Alternatively, they are Fusarium virulent genes which expressed specifically due to the interaction between Fusarium and Sumai 3. We are conducting northern and southern blots, trying to discriminate these two possibilities with these ESTs as probes.

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