

**U.S. Wheat and Barley Scab Initiative
 FY02 Final Performance Report (approx. May 02 – April 03)
 July 15, 2003**

Cover Page

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Year:	FY2002 (approx. May 02 – April 03)
Grant Number:	59-0790-1-078
Grant Title:	Fusarium Head Blight Research
FY02 ARS Award Amount:	\$ 58,084

Project

Program Area	Project Title	USWBSI Recommended Amount
BIO	Cloning and analysis of Fusarium and wheat genes essential to FHB development and resistance in wheat.	\$40,000
VDUN	Implementation of Marker-assisted Selection in the Scab Breeding and Germplasm Enhancement Programs in South Dakota.	\$19,536
	Total Amount Recommended	\$59,536

Principal Investigator

Date

Project 1: Cloning and analysis of Fusarium and wheat genes essential to FHB development and resistance in wheat.

1. What major problem or issue is being resolved and how are you resolving it?

The objectives set in our FY2002 proposal were: 1) continuing our screening for and cloning of differentially expressed ESTs of interest, 2) genetically confirming the association of the cloned ESTs with FHB or FHB-resistance and their potential usage as markers in breeding; 3) cloning the full-length cDNAs of the genes represented by the ESTs of interest with 5' race technology and 4) analyzing the sequences of the full-length cDNAs to reveal their potential functions. When the grant money was received in May, 2002, we had confirmed FHB-resistance associated ESTs C4, C7, A7, G12 and G75. Of the five ESTs, C4, C7 and G75 belong to *Fusarium graminearum*, and A7 and G12 represent wheat genes. Therefore, we adjusted our research emphasis to focus on further molecular study of the genes represented by these five ESTs. As a result, no further DDRT-PCR screening was conducted, and genetic analysis was also postponed until more advanced inbred population is available.

By the end of the FY2002 grand period, full-length mRNA sequences had been disclosed for C4, C7, G12 and G75 with the aid of the 5' RACE technique, and their full-length cDNAs had been cloned. Bioinformatic analysis of the full-length cDNAs revealed that C4 encodes a putative long-chain acyl-CoA binding protein; G75 encodes a protein that is 67% similar with a hypothetical protein discovered in *Neutospora crassa*; and the transcript of the cognate G12 gene is possibly a non-coding RNA. The C7 RNA was found to be homologous to *Fusarium* 18S rRNA.

We also carried our study further beyond our set objectives. The coding regions of the C4 and G12 genes were cloned from *F. graminearum* or wheat genome, respectively, and sequenced. No intron was found in the G12 coding region, and two introns were discovered in the C4 coding region. Searching GenBank databases also revealed that the cognate G75 gene does not have an intron in its coding region, too. In order to find the regulatory sequences, chromosomal walk was carried out up to 2 kb upstream of the C4 coding region. Promoter detection analysis indicated that this 2kb sequence was sufficient for the gene expression. Sequence analysis of this promoter region revealed one putative MRE-like cis-acting element located at 17 bp of upstream of the transcriptional starting site.

2. What were the most significant accomplishments?

- a. Cloned and analyzed the full-length cDNAs of four FHB-associated genes, including one wheat genes (*G12*) and three *Fusarium graminearum* genes (*G75*, *C4* and *C7*)
- b. Cloned and analyzed the coding regions of the cognate *G12*, *G75* and *C4* genes
- c. Cloned and functionally analyzed the 5' regulatory region of the cognate *C4* gene.

Project 2: Implementation of Marker-assisted Selection in the Scab Breeding and Germplasm Enhancement Programs in South Dakota.

1. What major problem or issue is being resolved and how are you resolving it?

Ninety-four hard red spring wheat lines and 61 hard red winter wheat lines from South Dakota wheat breeding programs were assayed for the FHB-resistance QTL *Qfhs.ndsu-3BS* with SSR primer sets *Xgwm389*, *Xgwm493*, and *Xgwm533*. The lines were selected from South Dakota Crop Performance Testing (CPT) and Advanced Yield Trial (AYT). All the spring wheat lines were selected based on their FHB resistance. The winter wheat lines were selected because their potential utility in the breeding program. SSR markers *Xgwm389*, *Xgwm533* and *Xgwm49* were chosen because they are among the most closely linked to the FHB-resistance QTL *Qfhs.ndsu-3BS* (Liu and Anderson, 2003, Crop Sci. 43:760-766).

For the spring wheat, 11 (11.7%) out of the 94 lines have the *Xgwm389-135* marker. Forty-five wheat lines (47.9%) have the *Xgwm493-190* marker. Twenty-three wheat lines (24.5%) have either the *Xgwm533-98* or the *Xgwm533-145*. Of the four markers, *Xgwm389-135* was the most polymorphic. An *Xgwm533-120* allele, which was a diagnostic marker for stem rust resistance gene *Sr2*, was observed in 62 (68.1%) spring wheat lines.

For the winter wheat, 22 lines have either the *Xgwm533-98* or the *Xgwm389-135* marker. However, none of the 61 winter lines was found to have the *Xgwm493-190* and the *Xgwm533-145*. Of the four markers, *Xgwm389-135* was also the most polymorphic. The *Xgwm533-120* allele relation to *Sr2* was observed in 35 (57.4%) winter wheat lines.

2. What were the most significant accomplishments?

Our assay disclosed that 53% of the advanced SD spring wheat lines we assayed have at least one of these two markers. Of those SD lines with the FHB resistance from Sumai 3, 68.4% have at least one of the markers. These results can be regarded as an estimate of the MAS efficiency if these two SSR markers were used as the sole selection criterion. None of the winter wheat lines tested have either marker.

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Xing, D.-H., Y. Yen and Y. Jin. 2002. A non-coding wheat RNA may play an important role in wheat resistance to Fusarium head blight. In: 2002 National Fusarium head Blight Forum Proceedings, Erlanger, KY, Dec 7-9, pp49.

Xing, D.-H., Y. Yen and Y. Jin. 2002. A putative acyl-CoA-binding-protein of *Fusarium graminearum* may play an important role in the FHB pathogenesis in wheat. In: 2002 National Fusarium head Blight Forum Proceedings, Erlanger, KY, Dec 7-9, pp50.

Liu, D.C., Y. Yen and A.M. Ibrahim. 2003. Screening elite South Dakota winter wheat for SSR markers linked to Fusarium head blight resistance. Proc. SD Acad. Sci. (accepted)

Xing, D.-H., Y. Yen, Y. Jin and J.C. Rudd. Expressed sequence tags specifically expressed during the initiation of the Fusarium head blight pathogenesis in FHB-resistant wheat. Theor. Appl. Genet. (Submitted)

Liu, D.C., Y. Yen and K. Glover. Evaluation on *Qfhs.ndsu-3BS* QTL for head scab with SSR markers in spring wheat breeding. Crop Sci (submitted)