USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY06 Final Performance Report (approx. May 06 – April 07)
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Cover Page

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USDA-ARS Agreement Title:	Saturation Mapping of the Chromosome 2(2H) Fusarium Head Blight Resistance QTL.
FY06 ARS Award Amount:	$ 57,372

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Award Amount
HGG	Fractional Analysis of Chromosome 2(2H) Fusarium Head Blight Resistance QTL.	$ 57,372
	Total Award Amount	**$ 57,372**

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Principal Investigator Date

* CBCC – Chemical, Biological & Cultural Control
EEDF – Etiology, Epidemiology & Disease Forecasting
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GET – Genetic Engineering & Transformation
HGR – Host Genetics Resources
HGG – Host Genetics & Genomics
PGG – Pathogen Genetics & Genomics
VDUN – Variety Development & Uniform Nurseries

Project 1: *Fractional Analysis of Chromosome 2(2H) Fusarium Head Blight Resistance QTL.*

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

Fusarium Head Blight remains a serious threat to the production and utilization of barley. Classical breeding has not been able to solve the problem to date. To assist classical breeding efforts we are saturating the chromosome 2H Bin 10 region (containing the major FHB resistance QTL in CI4196) with molecular markers that can be used in molecular marker assisted breeding and eventually for cloning of the FHB resistance gene(s). In addition, we have developed isolines that incorporate the 6-rowed (desired for breeding purposes) trait in FHB resistant material. Some of this material, but not all, also has improved maturity and reduced height compared to CI4196. Finally, we are using a mutagenesis approach to develop CI4196 lines with improved agronomic qualities such as reduced height and earlier maturity.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishment:

Research results:

The genetic and physical map in the chromosome 2H *Vrs1* region, roughly from BF263615 to MWG882, is now well saturated with molecular markers and we have identified multiple BAC clones. In order to saturate this region with markers, 29 rice chr. 4 bacterial artificial chromosome (BAC) clones with synteny to this region were blasted against the barley expressed sequence tag (EST) database. Currently, 80 markers at 31 unique loci are associated with this region. Of these, 46 have been hybridized to the 6x cv. Morex BAC library and 37 have identified positive BAC clones giving us a physical map consisting of 200 clones.

To establish a contiguous physical map of the chromosome 2H bin 10 region, we have identified our clones within the BAC clone contigs in BAC fingerprint database of the Tim Close lab at the University of California, Riverside (http://phymap.ucdavis.edu:8080/barley/index.jsp). This resulted in the identification of 6 BAC clone contigs in the region from *Vrs1* to MWG503. These BAC contigs contain a total of 106 clones covering a cumulative distance of 2.629 Mb. There remains a significant gap in the *Vrs1* distal region from marker BI955972 to MWG503. The reason for this gap is not known, but it could be due to the presence of a 5S RNA locus in this region. The 5S RNA locus presumably contains tandem repeats of the gene, which may facilitate recombination. Another possibility is that there are no genes in this region due to the 5S RNA locus. Other explanations, however, are also possible.

To obtain additional markers, we mapped 378 DArT markers on the Foster x CI4196 map (unpublished). This map was merged with the existing Foster x CI4196 map and with other DArT barley maps developed by Andrzej Kilian's group resulting in a highly marker enriched barley genome map (Wenzl *et al.*, 2006). However, the DArT markers also failed to fill the chromosome 2H bin 10 marker gap mentioned above.

Approximately 1 lb of CI4196 seed (from Rich Horsley) was irradiated with 4.5 Gy fast neutrons last spring and grown at Pullman WA (summer '05). Individual M1 heads and bulk M2 seed were harvested (summer '05). A bulk M2 field was grown at Pullman, WA (summer '06) and screened for morphological mutants. Jerry Franckowiak spent a few days at Pullman to help look for mutants. Some potentially useful mutants identified include 6-rowed, semi-dwarf, early maturity, lax spike, and upright spike. These have been confirmed as CI4196 based on

molecular markers at seven unique loci and will be BCed and mapped to determine if they are from the target region. They will also be tested for FHB resistance by Rich Horsley this summer ('07). Mutants of interest will be examined for hybridization to the Barley 1 microarray to identify deleted genes.

Seventy-four recombinants were identified from a Morex x FosCIA28 and Morex x FosCIA80 BC2F2 populations. The chromosome 2H bin 8-11 region was genotyped and seed sent to Dr. Rich Horsley for phenotyping in China. Results showed that some of the recombinants were 6-rowed, reduced height, earlier than CI4196 and with good FHB resistance. These lines will be retested for FHB tolerance and growth habit in North Dakota summer '07 by Rich Horsley. The recombinants were also planted in North Dakota summer ’06, but due to low FHB infection, no data are available. Previous data suggested that the region between the *Vrs1* locus and the proximal marker ctg9802, a distance of approximately 10-15 cM, is not essential for FHB resistance. This fragment was backcrossed into CI4196 to produce 6-rowed lines with CI4196 genomic background. Twenty nine of these recombinants were tested in China winter '06-'07 by Rich Horsley. All lines were 6-rowed, but tall and late. Two lines showed remarkable FHB tolerance (1.67-1.94) and several showed moderate tolerance. These lines will be retested in North Dakota summer '07 by Rich Horsley.

Six new CAP markers were developed to facilitate screening of recombinants. These are for markers MWG865, MWG699, BG343659, BI954724, BG369432, and BF263615.
We have placed emphasis on identifying potential candidate genes for FHB resistance and morphological characteristics based on the rice genome sequence and the chromosome 2H location of the FHB QTL. These genes were identified from the rice syntenous region and mapped where possible. Sometimes we had to develop primers based on the rice sequence. Several of the interesting gene homologs, such as Far red impaired response, Myb transcription factors, Avr9-Cf9 elicitor, Ring Zn finger, Elicitor response gene 3, NBS-LRR-type, reductase protein, and auxin response factor 10 all mapped proximal to the *Vrs1* locus. Based on the preliminary analysis of the genome regions described above, we do not believe that this region is involved in FHB resistance. However, some of these genes could be involved in the CI4196 undesirable morphological traits such as tall and late. The Far red impaired response gene is a good candidate for these traits.

Impact:

The development of a saturated map of the barley chromosome 2H bin 8-10 region affords the choice of many molecular markers for breeders and geneticists to select from. We have converted a number of the molecular markers into CAP markers to further facilitate their use. These are all available to the barley community. The development of recombinants with various chromosome 2H segments resulted in the development of 6-rowed lines with excellent FHB resistance. Some of the lines also have reduced height an improved maturity dates compared to the original resistant parent CI4196. These lines are available to the barley community. The map-based cloning of the FHB resistance gene(s) is a long term project. The work we have accomplished to date is moving us closer to that goal.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?

The scientific community has benefited from our genetic and physical mapping efforts by having a better understanding of the chromosome region involved in the FHB resistance and by gaining an appreciation of its DNA size. The development of a complete or even a partial physical contig allows the scientific community to plan to sequence the region in order to facilitate gene discovery. With the advancing DNA sequencing technology, even large DNA segments can be sequenced efficiently and with reasonable cost. Agriculture has benefited by our development of 6-rowed FHB resistant lines. These are excellent material for further breeding and they should hasten the development of FHB resistant cultivars. The mutants we have developed and are continuing to develop will also provide superior starting material for development of agronomically acceptable FHB resistant cultivars.

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.

Boyd, C., Maier, C., Sushailo, S., Horsley, R. and Kleinhofs, A. (2006) "Genetic and physical mapping of the barley chromosome 2(2H) Vrs1 region Fusarium Head Blight resistance QTLs." In: Canty, S. M., Lewis, J., Siler, L. and Ward, R. W. (Eds), Proceedings of the 2006 National Fusarium Head Blight Forum; Dec. 10-12, 2006; Research Triangle Park, North Carolina. Okemos, MI 48864 pp. 87-90.

Wenzl, P., H. Li, J. Carling, M. Zhou, H. Raman, E. Paul, P. Hearnden, C. Maier, L. Xia, V. Caig, J. Ovesna, M. Cakir, D. Poulsen, J. Wang, R. Raman, K. Smith, G.J. Muehlbauer, K.J. Chalmers, A. Kleinhofs, E. Huttner, and A. Kilian. 2006. A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7:26.