USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY05 Final Performance Report (April 05 – April 07)
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Cover Page

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Fiscal Year:	2005
FY05 ARS Agreement ID:	59-0790-5-F093
FY05 Agreement Title:	Novel FHB Resistance Sources in Bread Wheat from International Genetic Resources.
FY05 ARS Award Amount:	$ 80,000

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
GIE	Novel FHB Resistance Sources in Bread Wheat from International Genetic Resources.	$ 58,537
GIE	Novel Sources of FHB Resistance in Durum Wheat Through Use of Wild Relatives.	$ 21,463
	Total Award Amount	**$ 80,000**

Principal Investigator Date

* BIO – Biotechnology
CBC – Chemical & Biological Control
EDM – Epidemiology & Disease Management
FSTU – Food Safety, Toxicology, & Utilization
GIE – Germplasm Introduction & Enhancement
VDUN – Variety Development & Uniform Nurseries

Project 1: *Novel FHB Resistance Sources in Bread Wheat from International Genetic Resources.*

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

The most potent sources of resistance available to US FHB researchers derive primarily from a single source, Sumai 3. The heavy reliance on a single source of resistance is problematic as it presents risks including breakdown of the resistance, potential problems with linkage drag associated with the resistance, and because Sumai 3 alone does not provide adequate resistance under conditions of heavy epidemics. CIMMYT is resolving this problem by searching for other sources of resistance through the evaluation of genetic resources and the exchange of elite and research germplasm among FHB researchers around the world.

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 (1)

FHB Field Evaluation:

The change of our primary screening site from Toluca, Mexico, to El Batan, Mexico and the use of the newly identified *F. graminearum* isolates proved to be successful. In 2006, over 9,500 plots were planted in El Batan, Mexico, under artificial inoculation and misting for FHB evaluation. These entries were screened using spray inoculation (though selected nurseries were also point inoculated) with inoculum consisting of a mixture of three *F. graminearum* isolates (confirmed as lineage 3, Robert Bowden and John Leslie, KSU). Materials evaluated under artificial inoculation included, but were not limited to:

- Preliminary screening of advanced materials from the irrigated and rainfed CIMMYT wheat breeding programs
- Preliminary screening of synthetic derivatives and wide crosses
- Replicated screening of multiple mapping populations.
- Replicated screening of advanced FHB resistant materials.
- Replicated screening of one (1) resistant (Sumai 3) and three (3) susceptible checks throughout the trials.

At El Batan disease symptoms were evaluated at 31 days post inoculation, and data revealed that a wide range of symptoms could be observed between genotypes, as can be seen from the statistics of our preliminary screening trials (Table 1):

Table 1: Preliminary screening trial statistics of 3,530 entries (not including checks)

Disease Measure	Maximum (not including checks)	Minimum (not including checks)
FHB Incidence	100%	11.11%
FHB Severity	88.95%*	4.82%
FHB Index	88.95% *	0.58%**

*Notes were only taken on some selected susceptible lines for comparison – therefore higher levels may have been present.
**The average % FHB Index of Sumai 3 across 51 plots = 3.99 (SD=1.89)

Approximately 10% of preliminary screening entries (~350 entries) showed an FHB index of <10%. These were selected for further FHB screening and were planted in replicated trials in 2007.

In 2006, replicated screening (2-3 reps) was conducted on 243 advanced lines – including advanced FHB screening materials as well as elite breeder lines which had been evaluated for FHB over at least one year in Toluca. Thirty-one of these entries showed encouraging levels of resistance, and many of these lines did not have an obvious Chinese parent in their pedigree (i.e., resistance is less likely to be from the 3BS QTL). These 31 lines were recommended to CIMMYT breeders for further use/advancement and will be evaluated again for FHB resistance in 2007. Several of these lines were used in crossing (see below). In addition, these lines are being prepared for international distribution, though the number of lines distributed may be further reduced depending on additional data.

Impact (1):
We have an effective screening site/method for differentiating between resistant and susceptible genotypes and the capacity to screen thousands of genotypes on an annual basis. We have identified many good sources of resistance, and many of these sources may be different from 3BS.

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? (1):
Evidence of improved screening facilities in Mexico for the identification of resistant materials and other collaborative projects. Access to new germplasm that has been identified as resistant.

Accomplishment (2)
Fusarium International Spring Wheat Nurseries:
CIMMYT has initiated, through the collective request and endorsement by the participants of the "CIMMYT Workshop on the Global Fusarium Initiative for International Collaboration" (March 2006), multi-origin international spring wheat nurseries that involve the active participation of top FHB researchers around the world. Two nurseries are being compiled with the following description:

1. Fusarium International Elite Spring Wheat Nursery (FIESWN).
The specific objective of this nursery is to enable contributors to know the performance of their entries across environments, and allow participants to identify useful sources of resistance in entries from other programs. The nursery includes two types of entries:

- Elite FHB/FCR resistant spring wheat (registered or near-registered resistant cultivars) that have performed well in regional FHB/FCR nurseries.
- Regional FHB/FCR resistant and susceptible reference/standard checks.

2. Fusarium International Preliminary Spring Wheat Nursery (FIPSWN).
The purposes of this nursery include: identification of new sources of resistance; examination of stability of QTL for FHB/FCR resistance; surveillance for new and/or problematic pathogen strains; and, development of knowledge or solutions in regard to other issues such as negative correlations between resistance QTL and other traits. The nursery can include:

- Any materials which address the objectives listed above including NILs of FHB/FCR QTL; parents of mapping populations;

The overall objective of these two nurseries is to make useful materials for Fusarium Head Blight (FHB) and Fusarium Crown Rot (FCR) available throughout the world. CIMMYT is serving as the coordinator of these two nurseries. CIMMYT routinely develops internationally distributed nurseries. A time-line for the annual development of the nurseries can be seen in Figure 1. The increase of the first year's entries have been recently received from Mexicali (our Karnal bunt-free site in northern Mexico), and will be distributed to cooperators later this year. Many entries from the 1st and 2nd sets of nurseries are also planted for FHB evaluation in El Batan this summer. A summary of the institutions and corresponding countries participating can be seen in Table 1. To date, we have received 188 entry nominations for these nurseries (1st and/or 2nd FIESWN and FIPSWN).

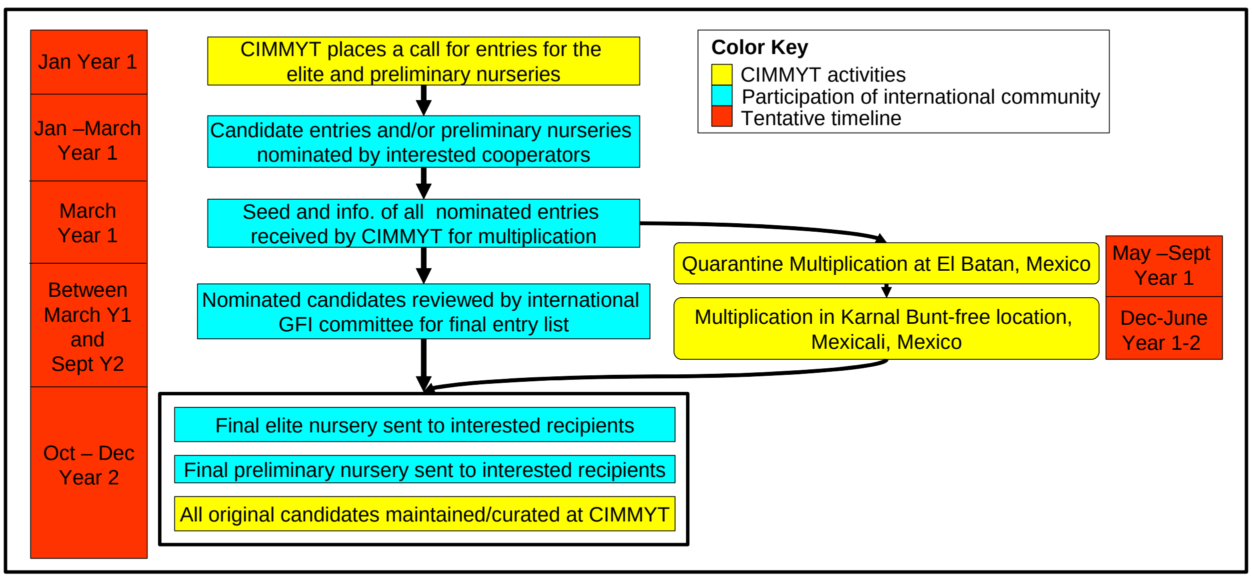

Fig 1: Timeline for annual preparation and distribution of Fusarium International Elite and Fusarium International Preliminary Spring Wheat Nurseries. (Y1 = Year 2, Y2 = Year 2, GFI = Global Fusarium Initiative)

Table 1: Current list of the countries of participating institutions for the 1st and/or 2nd FIESWN and/or FIPSWN.

Country	Institution
Argentina	Buck Semillas
Austria	Institute for Plant Production

Brazil	OR Melhoramento de Sementes Ltda.
Canada	Agriculture and Agri-Food Canada (multiple cooperators)
Germany	Lowchow-Petkus
Inia La Estanzuela	Uruguay
Iran	Seed and Plant Improvement Institute (SPII)
Mexico	CIMMYT
South Africa	ARC-Small Grains institute
Switzerland	Agroscpe Changins-Wadenswil
Turkey	CIMMYT
United Kingdom	National Institute of Agricultural Botany
United States of America	Michigan State University
United States of America	University of Minnesota
United States of America	Colorado State University/Dakota Growers Pasta Company

Impact (2)

These nurseries will enable the validation of various resistance sources by multiple FHB programs around the world, mutual awareness in the FHB community of available materials, and streamlined deployment of valuable germplasm and corresponding knowledge to FHB breeders and researchers world-wide.

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?

FHB researchers around the globe have a central exchange and access point for FHB resistant germplasm and data regarding this germplasm, irrespective of its origin.

Accomplishment (3)

Toxin Evaluation

The most critical limiting factor regarding FHB faced by farmers and the industry today is the accumulation of toxin in the grain. Hundreds of samples have been quantified for DON toxin at CIMMYT in the past year using the RIDASCREEN®FAST DON ELISA kit, and the results have shown a wide range of toxin accumulation in the samples (from <0.222ppm to >6ppm – the limits of accurate detection with the kit). These samples have included breeding materials, a survey group of resistant and susceptible bread wheat, durum wheat and barley, mapping populations, and samples from long term trials of varied cultural practices. Additional samples are also being planned for toxin evaluation including advanced FHB and breeding materials, elite materials from the Southern Cone (using a fluoroquant method), and other specific research studies.

Impact (3)

Toxin data is being used to help identify of the most promising lines for resistance to make available to international cooperators and to also use in CIMMYT's breeding programs.

<u>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?</u>
Breeders and other users of germplasm screened at CIMMYT will have critical toxin accumulation data available regarding the germplasm that they decide to obtain from CIMMYT (or for their own germplasm if they are cooperating with CIMMYT for screening). This knowledge empowers breeders and users to make more effective selections of germplasm to reduce the occurrence and impact of FHB.

Accomplishment (4)
Quantitative PCR (qPCR) for Toxin Analysis
Over the past year, in addition to using ELISA methods for toxin quantification, quantitative PCR of fungal DNA was tested for correlation with toxin production. Quantitative PCR is lower in cost than ELISA kits and uses a smaller sample volume. Three different groups of materials (two (2) mapping populations and a survey group of 64 bread wheat, durum and barley lines) were used to test the correlation of immunoassay vs. quantitative PCR results. The results showed that there was a significant correlation between toxin (DON) and fungal biomass.

<u>Impact (4)</u>
qPCR can be proposed as a first-screening of mycotoxin contamination for discarding the entries with higher toxin contamination.

<u>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?</u>
CIMMYT as well as other FHB researchers have evidence that qPCR may be useful as an option for an initial screening of relative levels of toxin contamination.

<u>Accomplishment (5):</u>
Validation of Seedling Bioassay
A live-leaf seedling bioassay has been developed for potential use as a first-cut screening method for identification of genotypes for resistance/susceptibility to FHB. This method is rapid, requires little space, and can be conducted any time of the year. A survey group of 64 bread wheat, durum and barley lines with different genetic backgrounds and levels of resistance to FHB were evaluated in a 3-replication experiment in the field, and were also examined with the seedling bioassay to test the validity of the bioassay. A significant correlation was confirmed between lesion size on the seedling (after inoculation with *F. gramineraum* spores) and the field FHB phenotypic data. These results suggest that common resistance factors were induced in both seedlings and spikes and the new bioassay system can be useful for the first screening test of FHB resistance germplasm.

<u>Impact (5):</u>
A new seedling bioassay has shown good correlation with field symptoms at CIMMYT.

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 seedling bioassay can be considered for preliminary rapid screening of genotypes.

Accomplishment (6)

Crosses:

Thirty-six of the best performing entries, according to replicated evaluations in 2006 and previous evaluations, were selected and made available to the CIMMYT irrigated and rainfed bread wheat breeding programs to use in their crossing blocks. In addition, focused crosses were also conducted between the selected best materials for the FHB program for the purpose of pyramiding resistances from different resistance sources. Some of these sources have been previously mapped (i.e., Sumai 3, Nobeokabozu komugi, and a synthetic derived line developed at CIMMYT), and some of these sources are not yet mapped. F1's of the crosses for the FHB program are also being planted in a crossing block in 2007 for developing top-crosses for some of the crosses. F2 populations will also be generated. In addition, selected resistant sources which have not been previously mapped will be crossed with susceptible genotypes for the preparation of di-haploid populations to map novel sources of resistance from the best materials identified at CIMMYT.

Impact (6)

Strong sources of resistance will be pyramided for more effective resistance to FHB. New sources of strong resistance will be mapped.

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?

Germplasm with multiple QTLs for resistance, including some likely novel sources of resistance. Mapping information regarding new sources of strong resistance will be available, which can be used with marker assisted selection.

Project 2: *Novel Sources of FHB Resistance in Durum Wheat Through Use of Wild Relatives.*

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

FHB breeding in durum remains problematic because of the lack of potent resistance genes. We are resolving this problem through: i) introduction of resistance from other relevant genotypes in cytogenetic stocks; ii) screening elite durum wheat containing the *Fhb1* allele for resistance on chromosome arm 3BS of the hexaploid wheat; iii) examination of CIMMYT genebank materials.

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 (1):

New genetic resources of durum wheat, potentially carrying resistances from hexaploid and diploid relatives have been created or are being prepared for screening for resistance.

(1) F2 plants of crosses between *ph1c* bearing durum wheat and two putatively resistant *Aegilops tauschii* accessions have been developed. These plants were subjected to cytogenetic analysis to identify those carrying translocations from the *Aegilops* donor. To date, the first 30 sets of seeds have been examined cytogenetically, but no translocation was identified.

(2) Over seventy (70) BC4F2 durum wheat lines selected (using markers) for the *Fhb1* QTL as well as leaf and yellow rust resistance were created (*work funded by a different granting agency*) and have been been planted in El Batan for FHB evaluation in 2007.

Impact (1):

New genetic resources with potential FHB resistance have been generated in durum wheat.

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?:

Durum wheat with potentially new levels of FHB resistance.

Accomplishment (2):

Screening genetic resoureces

Eighteen (18) accessions of *Triticum turgidum* var *carthlicum* (AABB) were acquired from the CIMMYT genebank and screened for FHB resistance in El Batan in 2006. Unfortunately, none of these genotypes demonstrated good levels of resistance to FHB. In 2007, 27 A-genome synthetics (AAAABB) and 24 B-genome synthetics (AABBBB) created at CIMMYT were planted for FHB evaluation in El Batan. In addition, six durum x alien species amphidiploids were planted for evaluation.

Impact:
Although good sources of resistance were not found in the carthlicum accessions evaluated in 2006, additional A and B genome sources of resistance may be identified from germplasm that will be evaluated in 2007.

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?:
Potentially new sources of A and B genome resistance.

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.

Proceedings and Presentations;

Ban, T., Lewis, JM. and Ward, RW. (2006) Global Fusarium Initiative for International collaboration on breeding and host plant resistance to Fusarium Head Blight. The 9th European Fusarium Seminar, 19-22 September 2006, Wageningen, The Netherlands, 85.

Murakami, J. and Ban, T. (2006) A novel seedling bioassay for Fusarium fungus pathogenicity and toxigenicity causing Fusarium head blight. The 9th European Fusarium Seminer, 19-24th September, Wageningen, the Natherlands.

Murakami, J. and Ban, T. (2006) Development of simple bioassay system for pathogenicity and toxigenicity of Fusarium fungus causing Fusarium head blight of wheat. Advances on genomics, biodiversity and rapid systems for detection of toxigenic fungi and mycotoxins, 26-30th September, Bari, Italy.

Lewis, J.L., Velazquez, C., Murakami, J., Capettini, F., Ban, T., Ward, R.W. (2006) Facilitation of International Fusarium Nurseries and Improvements of FHB Screening System at CIMMYT. In: Proceedings of the 2006 National Fusarium Head Blight Forum; 2006 Dec 10-12; Research Triangle Park, NC. East Lansing: Michigan State University, p. 109.

Ban, T. (2006) Global Fusarium Initiative for International Global Collaboration of Genetic Studies and Breeding for Fusarium Head Blight Resistance in Wheat and Barley. In: Ban, T., Lewis, J.M. and Phipps, E.E. (eds.) The Global Fusarium Initiative for International Collaboration: A Strategic Plannning Workshop held at CIMMYT, El Batan, Mexico; March 14-17, 2006. Mexico, D.F.: CIMMYT, pp. 1-2.

Kishii, M., Delgado, R., Rosas, V., Cortes, A., Cano, S., Sanchez, J., Mujeeb-Kazi, A., Lewis J., and Ban, T. (2006) Utilization of Wild Genetic Resources for the Improvement of FHB resistance in Wheat Breeding. In: Ban, T., Lewis, J.M. and Phipps, E.E. (eds.) The Global Fusarium Initiative for International Collaboration: A Strategic Plannning Workshop held at CIMMYT, El Batan, Mexico; March 14-17, 2006. Mexico, D.F.: CIMMYT, pp. 24-27.

Ortiz, R., Ban, T., Bandyopadhyay, R., Banziger, M., Bergvinson, D., Hell, K., James, B., Jeffers, D., Kumar, P. L., Menkir, A., Murakami, J., Nigma, S. N., Upadhyaya, H. D., and Waliyar, F. CGIAR research-for-development program on mycotoxins. Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. (in press).

Shi, F., Xu, D., Handa, H. and Ban, T. (2006) Developing DNA markers associated with QTLs for low mycotoxin accumulation and/or Fusarium Head Blight resistance based on the information of ESTs induced by Fusarium mycotoxin in wheat. Breeding Research 8 (supl. 1), 56 (in Japanese).

Yu, J-B., Bai, G-H., Cai, S-B. and Ban, T. (2006) Marker-assisted characterization of Asian wheat lines for resistance to Fusarium head blight. Theoretical and Applied Genetics 133 (2): 308-320.

Shi, F., Elouafi, I., Xu, DH., Handa, H. and Ban, T. (2006) Toward understanding the genes related to QTLs for Fusarium Head Blight resistance. The 9th European Fusarium Seminar, 19-22 September 2006, Wageningen, The Netherlands, 113.

Shi, J.R., Yang, H., Lu, Q., Xu, D. and Ban, T. (2006) DNA Marker Analysis for FHB-Resistance Pyramiding from Different Genetic Germplasms. In: Ban, T., Lewis, J.M. and Phipps, E.E. (eds.) The Global Fusarium Initiative for International Collaboration: A Strategic Plannning Workshop held at CIMMYT, El Batan, Mexico; March 14-17, 2006. Mexico, D.F.: CIMMYT, pp. 30.