USDA-ARS / USWBSI
FY04 Final Performance Report
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Cover Page

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Year:	**FY2004** (approx. May 04 – April 05)
FY04 ARS Agreement ID:	**59-0790-4-092**
FY04 ARS Agreement Title:	**Developing Winter Wheat with Improved Fusarium Head Blight Tolerance by Conventional and Transgenic Approaches.**
FY04 ARS Award Amount:	**$ 106,218**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	Enhanced Scab Resistance in Winter Wheat Germplasm by Plant Transformation.	$ 58,605
VDUN	To Enhance Variety Development of Scab Resistant Varieties.	$ 47,613
	Total ARS Award Amount	**$ 106,218**

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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: ***Enhanced Scab Resistance in Winter Wheat Germplasm by Plant Transformation.***

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

There are two major objectives required for success of this research project which entails expression of anti-apoptotic genes as a means to enhance scab resistance: (i) Wheat transformation/ stable gene expression and (ii) reliable field testing of transgenic wheat. Importantly, both of these issues have been met. We have been successful in generating 70 events in wheat including the generation of lines harboring Bcl-2, CED-9, Op-IAP, Sf-IAP and a non-coding RNA; all of which have been shown to suppress cell death in other plant species as well as mammalian cells. An additional 18 events for ribosome inactivating protein or a lytic peptide have been created. We have obtained stable lines of wheat expressing all of these constructs using Agrobacterium tumefaciens mediated transformation and have identified numerous stable lines after multiple generations of selfing (e.g. F_7 plants for Bcl-xL transformed wheat). Thus problems associated with gene silencing and instability of foreign DNA have been overcome and as a result we have stable lines expressing all of our transgenes for field testing. In order to get a comprehensive evaluation of transgenic field performance, we have implemented field trials in a number of locations both in Nebraska (2) as well as with Dr. Mohammed Mergoum in North Dakota (1 testing site). We have also collaborative greenhouse trials with Dr. Yue Jin, USDA-ARS, St. Paul, Minnesota. Thus transgenic plants will face different environmental conditions and especially one in the spring wheat-growing region where scab is a major problem. As a result of developing field-testing "infrastructure", we are now in an excellent position to validate the concept of cell death modulation as a novel approach for scab resistance.

2. What were the most significant accomplishments?

As discussed in the preceding section, our most significant accomplishment is addressing the issues of stable wheat transformation and establishing a range of field sites to test our transgenic wheat. (Proof of concept)

In addition, we have established that deoxynivalenol (DON), a major constraint to wheat production, induces a programmed cell death in wheat (in situ, in vitro), with features similar to mammalian. A characteristic increase in TUNEL staining, ROS production and membrane disassociation are all noted following exposure of wheat tissues to DON.

Project 2: ***To Enhance Variety Development of Scab Resistant Varieties.***

1. What major problem or issue is being resolved and how are you resolving it?
The major issue that we are trying to resolve is the creation of scab tolerant lines using conventional plant breeding. We continue to use parent lines involving the Sumai 3 and other Chinese or derived line resistance, as well as, numerous lines that have other sources of resistance, such as soft wheats: Roane, Goldfield, and IN92823a1-1-4-4-5. Fifty-seven lines from these materials have entered our local scab-screening nursery based on agronomic appearance. These lines are being screened this summer and we are waiting for the results. In addition, we continue to work with Dr. Anne McKendry to incorporate resistance from new winter wheat sources from her screening program. She sent us numerous CIMMYT and Hungarian lines. Unfortunately, the Hungarian lines (which tended to have the better level of scab tolerance) appeared to be very poor parent lines and resembled Chinese Spring in our greenhouses. We crossed to every one of the Hungarian lines. As for the CIMMYT lines, we took a more opportunistic approach and crossed to those that appeared to have excellent plant types (mainly excellent floret fertility, large heads, and semi-dwarf stature with strong straw). Roane, and IN92823a1-1-4-4-5 seem to have good resistance to many of the diseases that are found in the field in Nebraska. Unfortunately, Goldfield is susceptible to stripe rust and many of the lines derived from crosses to Nebraska lines have led to very susceptible lines to stripe rust. The Goldfield type is a good type agronomically and the derived lines look good, but stripe rust has become a major disease in Nebraska, so the susceptible derivative lines will be dropped from further testing. Dr. Guihua Bai has screened our lines for the known QTLs on 3BS and the Goldfield QTLs (graciously provided by Dr. Herb Ohm). Interestingly, four advanced lines have the markers for the 3BS QTL. However, none of the lines have obvious parental ties to Sumai 3. If these markers are in the Nebraska material, it may complicate marker assisted backcrossing. None of the lines that express a higher level of scab tolerance identified in our field screens contain the 3BS QTL markers indicating they have the "native" resistance that others have found. The Goldfield QTLs were problematic in that they required the parental line to insure the correct bands were scored. IN addition, we gave Dr. Bai a population for his use in creating rapid backcross derived lines (similar to what the Canadian scab researchers are doing) that will be scab tolerant using the 3BS QTL. In our field trials, over 900 of the most advanced Nebraska and Great Plaines lines are currently being screened for resistance to scab. We continue to try novel screening methods and our advanced nursery was screened for resistance to scab, using a seed germination and detached leaf assay, by Dr Roy Browne.

2. What were the most significant accomplishments?
Wesley, a released line, has been identified as being superior for scab tolerance to other commercially available lines. It is currently grown on 5.5% of Nebraska's wheat acreage. More importantly it is primarily grown where scab is most common. Specifically it is grown on 18.6% of the southeast, 10.0% of the east, 18.7% of the central and 5.7% of the southcentral regions of Nebraska (most prone to scab infection). Arapahoe, which is grown on 5.2% of Nebraska's wheat acreage, also is believed to have a better scab tolerance than most lines. Arapahoe is grown on 3.6% of the southeast, 8.1% of the east, 13.0% of the central and 4.4% of the south central regions of Nebraska (most prone to scab infection). A new experimental line, NE01643, is being increased for possible co-release in Nebraska and South Dakota. It has superior scab tolerance also.

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 you grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Baenziger, P.S., M. Dickman, T. Clemente, S. Sato, A. Mitra, J. Watkins, B. Langston, B. LaVallee, J. Schimelfenig, S. Mitra, J. Bohlmann and M. Montgomery. 2004. Wheat Transformation: A needed tool for Wheat Genetics and Germplasm Improvement. Proceedings of the 2nd International Symposium on Fusarium Head Blight *incorporating the 8th European Fusarium Seminar*. Plenary Talk. pp 237.

Schimelfenig, J., P. S. Baenziger, and J. E. Watkins. 2004. The Development of Fusarium Head Blight (FHB) Tolerant Varieties of Wheat in Nebraska from 2001 to 2005. Proceedings of the 2nd International Symposium on Fusarium Head Blight *incorporating the 8th European Fusarium Seminar*. pp 156.