U.S. Wheat and Barley Scab Initiative Annual Progress Report September 15, 1999

Cover Page

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Year:	FY1999
Grant Number:	59-0790-9-026
Grant Title:	Fusarium Head Blight Research
Amount Granted:	\$69,283.00

Project

Program Area	Objective	Requested Amount
Biotechnology	Enhance scab resistance in winter wheat	\$35,000
	germplasm by plant transformation.	
Variety Development	To enhance variety development of scab	\$35,000
	resistant varieties.	
	Requested Total	\$70,000 ¹

Principle Investigator

Date

¹ Note: The Requested Total and the Amount Granted are not equal.

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Project 1: Enhance scab resistance in winter wheat germplasm by plant transformation.

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

We believe that the current germplasm resources available to create scab resistant wheat cultivars is limiting and often inherited in a complex manner. As such, our efforts have concentrated on transferring novel genes, from diverse sources, with known disease resistance activity into wheat using plant transformation. The four genes, we have attempted to insert into wheat encode: a) CED9, b) IAP (inhibitor of apoptosis), c) lactoferrin and a related derived protein, lactoferricin, and d) oxalyl-CoA- decarboxylase. We also wish to insert both lactoferrin and oxalyl-CoAdecarboxylase in hopes the two genes combined may have enhanced antifungal properties. These four genes were chosen because these genes in transgenic tobacco plants have shown potential for combating economically important fungal diseases of crop plants. In addition, these four genes represent distinctly different target specificities (modes of action). Our interest in CED9 and IAP is that host recognition of a pathogen triggers a cell death pathway resulting in a zone of dead cells around the infection site, a hypersensitive reaction. CED9 and IAP are known regulators of programmed cell death. Lactoferrin is a granule-associated glycoprotein present in mammalian fluids such as milk or tears that has long been reported as a major component of infant defense systems against microbial pathogens. Both lactoferrin and lactoferricin have been shown to be highly antifungal against yeasts and filamentous fungi at concentrations ranging from 3 to 25 µg/ml. Oxalyl-CoA-decarboxylase gene has been cloned from a soil bacterium to specifically degrade oxalic acid which is a pathogenic determinant of certain plant pathogenic fungi such as *Sclerotinia* and Rhizoctonia. Although it is not known if oxalic acid is involved in Fusarium pathogenesis, this gene might be helpful in providing resistance against the fungus by alteration of pH, chelation and/or neutralization. Our goal is to create 10 transgenic events for each gene we wish to insert.

2. Please provide a comparison of the actual accomplishments with the objectives established.

We have created 11 transgenic events for IAP (8 using Agrobacterium and 3 using microprojectile bombardment), 24 events for lactoferrin (using microprojectile bombardment), 10 events for oxalyl-CoA-decarboxylase (using microprojectile bombardment) and 18 events for lactoferrin and oxalyl-CoA-decarboxylase (using microprojectile bombardment). We were able to create only 2 transgenic plants for CED9. We are unsure of why we have a low frequency of CED9 transformants and will change the construct to see if perhaps there is something unusual with our construct. It is also possible that CED9 is deleterious to transgenic plans and few survive. Seeds from the most advanced lines are being grown in the greenhouse for screening for Fusarium head blight.

3. What were the reasons established objectives were not met? If applicable.

The only objective that is currently not met is the development of 10 transgenic CED9 plants. We do not know why this has occurred but are developing new constructs and have continued attempting to transform wheat with the gene. An equal number of embryos were used in the IAP and CED9 transformations, so the lack of transformation success is not due to having an insufficient number of embryos used in the transformation efforts to expect success.

4. What were the most significant accomplishments this past year?

The most significant accomplishments of the last year were to put three of our four target genes into wheat and to advance the generations of the advanced lines to a point where we can begin screening R_2 plants for Fusarium head blight resistance. We have also successfully adapted Agrobacterium transformation technology to be routinely used in wheat.

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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?

Fusarium head blight (commonly called scab) is a major problem in eastern Nebraska and the irrigated wheat production in western Nebraska. The most cost effective way to reduce losses to Fusarium head blight is to have cultivars that are resistant to the disease. We are attempting to breed Fusarium head blight resistant lines using conventional plant breeding techniques, specifically: 1. Collecting Fusarium head blight resistance germplasm, 2. Crossing Fusarium head blight resistant germplasm onto elite lines adapted to Nebraska, and 3. Screening the progeny of these crosses for Fusarium head blight resistance.

2. Please provide a comparison of the actual accomplishments with the objectives established.

We have collected Fusarium head blight resistant germplasm from Korea and the spring wheat breeding programs. We are especially grateful to Drs. Jackie Rudd and Yue Jin of South Dakota State University and to Dr. Bob Busch who have graciously shared their most resistant germplasm. Crosses were made onto this germplasm in our spring, 1999 crossing block. It is too early to screen the progeny of this material. In addition, one elite line which is believed to have a low level of Fusarium head blight tolerance (suggested by Dr. Yue Jin) was submitted to the Uniform Winter Wheat Scab Nursery for testing in 1999-2000

3. What were the reasons established objectives were not met? If applicable.

All of the objectives were met within the expected timeframe. Breeding by nature is a long-term enterprise.

4. What were the most significant accomplishments this past year?

Our most important accomplishment was the collection of the germplasm and the identification of a line that may already have a higher level of tolerance than many hard red winter wheat cultivar.

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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.

None during this period.