The 1998 National Fusarium Head Blight Forum

CHAPTER 4 Part 2

Host Resistance and Variety Development

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U.S. Wheat & Barley
Scab Initiative

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Wild Relatives of Wheat as Sources of Fusarium Head Blight Resistance

Prem P. Jauhar[1] and Terrance S. Peterson[2]

Abstract

Scab or Fusarium Head Blight (FHB) is a devastating disease of wheat. There is no reliable source of scab resistance in the current wheat cultivars. We have found that two wild relatives of wheat — the tetraploid wheatgrass (*Thinopyrum junceiforme*) and diploid wheatgrass (*Lophopyrum elongatum*) — are excellent sources of resistance to FHB. To transfer this resistance to durum wheat, we crossed two cultivars, Lloyd and Langdon, with the wild grasses. The synthetic Lloyd x *Th. junceiforme* F_1 hybrids had a meiotic association of 0.09 IV + 0.35 III + 7.27 II + 12.05 I, and were sterile. By backcrossing the sterile F_1 hybrids to Lloyd, we produced several fertile hybrid derivatives with the full chromosome complement of durum wheat plus a few chromosomes or chromosome segments of the *junceiforme* parent. Fluorescent *in situ* hybridization (FISH) analysis confirmed the integration of alien chromatin into the durum genome. Langdon x *L. elongatum* hybrid derivatives also showed integration of alien chromatin into the durum genome. Some of the Lloyd-*junceiforme* and Langdon-*elongatum* derivatives showed high resistance to FHB. With further backcrossing and selection it should be possible to produce durum cultivars with FHB resistance.

Introduction

Fusarium Head Blight (FHB), commonly called Scab, is caused by the fungus *Fusarium graminearum,* which infects wheat heads from flowering through grain fill, resulting in huge losses (approaching a billion dollars in the U.S. alone in some years) due to loss in grain yield and quality. There is no reliable source of scab resistance in current wheat cultivars. We have found that two wild relatives of wheat — tetraploid wheatgrass, *Thinopyrum junceiforme* ($2n = 4x = 28$; $J_1J_1J_2J_2$) and diploid wheatgrass, *Lophopyrum elongatum* ($2n = 2x = 14$; EE) — are excellent sources of resistance to scab. To transfer this resistance to durum wheat (*Triticum turgidum*; $2n = 4x = 28$; AABB), we hybridized two agronomically superior cultivars, Lloyd and Langdon, with the wheatgrasses. The synthetic Lloyd x *Th. junceiforme* F_1 hybrids ($2n = 4x = 28$; ABJ_1J_2) and the Langdon x *L. elongatum* F_1 hybrids ($2n = 3x = 21$; ABE) were sterile. By backcrossing these F_1 hybrids to their respective durum parents, we produced several fertile hybrid derivatives, some of which showed high levels of resistance to scab. Fluorescent *in situ* hybridization (FISH) showed integration of alien chromatin into the durum genome. Data on chromosomal studies and scab resistance of the hybrid derivatives are presented and their breeding significance discussed.

Materials and Methods

Synthesis of Hybrids

Hybrids between the durum wheat cultivar Lloyd and *Th. junceiforme* and between Langdon and *L. elongatum* were synthesized, using wheat as the female parents. The florets were manually emasculated and pollinated. One day after pollination, each floret was sprayed with a hormone solution containing GA_3 (75mg L^{-1}) and 2,4-D (5mg L^{-1}) (Jauhar and Peterson, 1996). The developing embryos were rescued by *in vitro* culture, on modified MS media, 10-16 days after pollination, and plants were grown to maturity.

Chromosome Studies

For meiotic analysis, spikes were fixed and anthers squashed as described by Jauhar (1991). At least 50 pollen mother cells (PMCs) were scored for meiotic configurations and chiasma frequency.

Fluorescent in situ *Hybridization (FISH)*

Anthers were squashed in 45% acetic acid and stored at –80 °C for up to 7 days; cover slips were removed using compressed CO_2. Slides were hybridized following the protocol of Islam-Faridi (pers. commun.,1996). Sheared genomic DNA of the grass parent was used as a probe, labeled with biotin-14-dATP.

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Autoclaved genomic durum wheat DNA was used as a blocker. The probe was detected using FITC-conjugated avidin; propidium iodide (PI) was used as a counter stain. Hybridized chromosome spreads were screened under a fluorescence microscope, using appropriate filter sets. Pairing was scored, and photographs taken using Kodak Ektachrome 100 ASA slide film.

Fusarium Screening

Plants were screened for response to FHB by inoculating two opposite florets in the middle of a spike with 10 *µl* inoculum (50,000 spores / *µl*) per floret. Three spikes were inoculated per plant. The plants were then misted with water and covered overnight, to maintain high humidity, and uncovered each day. This covering and uncovering procedure was conducted for 5 days after which the plants were uncovered and left to grow. Scoring was conducted two weeks after inoculation (Stack and McMullen, 1994). Plants which scored 33% or less infection were retained for further crossing and cytological analysis. Those with higher infection levels were discarded.

Results and Discussion

Hybrids between the durum wheat cultivar Lloyd and *Th. junceiforme* were sterile. Meiosis in the hybrids ($2n = 4x = 28$; ABJ_1J_2) showed a mean association of 0.09 IV + 0.35 III + 7.27 II + 12.05 I. Most of this pairing was between the J_1- and J_2-genome chromosomes; up to 7 bivalents were formed. However, some pairing occurred among the wheat chromosomes, i.e., between the chromosomes of the A and B genomes, and some between wheat and grass chromosomes. The formation of trivalents and quadrivalents involved wheat-grass pairing, which is a welcome feature from the breeding standpoint. Pairing between wheat and grass chromosomes was confirmed by FISH. The pairing resulted in the incorporation of alien chromatin into the wheat complement. FISH is an excellent tool for identification of parental chromosomes during meiosis in hybrids or polyhaploids and thus assessing their chromosome relationships (Schwarzacher et al., 1992; Heslop-Harrison and Schwarzacher, 1996; Mukai, 1996; Jauhar, 1997; Jauhar et al., 1997; Jauhar et al., in press).

Backcrossing F_1 hybrids with Lloyd yielded fertile progeny with 42 chromosomes, which resulted from fusion of unreduced gamete ($n = 28$) of the F_1 hybrid with a reduced gamete ($n = 14$) of the durum parent. Thus, these BC_1 derivatives had the genomic constitution $AABBJ_1J_2$, which was well reflected in the specificity of chromosome pairing, as revealed by FISH (Jauhar and Peterson, 1998). By further backcrossing these BC_1 progeny to Lloyd, we have produced some promising fertile hybrid derivatives. These derivatives have the full complement of the durum parent plus a few chromosomes or chromosome segments of the wild parent.

We have also produced promising, fertile hybrid derivatives from the cross Langdon x *L. elongatum*. These derivatives have some (presumably) desirable chromosome segments of the *L. elongatum* integrated into the wheat genome.

The fertile Lloyd x *Th. junceiforme* and Langdon x *L. elongatum* hybrid derivatives were screened for scab resistance. Some showed excellent resistance (Table 1) and are being further investigated. With further backcrossing and selection we expect to produce genetically stable, scab resistant durum cultivars. Wide hybridization followed by interspecific chromosome manipulation offers an excellent means of introducing scab resistance into durum wheat.

We are currently screening other relatives of wheat for inclusion in our *Fusarium* hybridization program. Some lines show promise for future development (Table 2).

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Table 1. Preliminary Fusarium Head Blight Screening Results of Hybrid Derivatives

Line	Number of plants (average of three spikes scored per plant) Percent infection 0-2	3-7	8-15	16-21	22-33	34-50	51-67	68-80	81-100	Total plants	Mean % infection of population
PRE-12[a]	0	3	13	8	10	18	7	3	3	65	33.20
PRE-13[b]	0	0	12	4	10	11	6	4	4	51	37.17
PRE-14[c]	0	0	8	3	6	15	12	10	13	67	51.34
PRE-15[d]	0	2	6	4	12	6	2	0	2	34	27.80
PRE-16[e]	0	6	16	3	11	5	4	0	2	47	24.79
PRE-17[f]	0	0	1	0	0	4	1	0	1	7	44.04
PRE-18[g]	0	0	0	2	1	1	1	1	2	8	53.02

[a] PRE-12 = Lloyd/*Th. junceiforme*//Lloyd F_3

[b] PRE-13 = Lloyd/*Th. junceiforme*//Lloyd F_3

[c] PRE-14 = Lloyd/*Th. junceiforme*//Lloyd F_3

[d] PRE-15 = Lloyd/*Th. junceiforme*//Lloyd F_3

[e] PRE-16 = Lloyd/*Th. junceiforme*//Lloyd F_2

[f] PRE-17 = Langdon/*L. elongatum*//Langdon F_2

[g] PRE-18 = Langdon/*L. elongatum*//Langdon F_2

Table 2. Preliminary Fusarium Head Blight Screening Results of Parental Grass Lines

Line	Number of plants (average of three spikes scored per plant) Percent infection 0-2	3-7	8-15	16-21	22-33	34-50	51-67	68-80	81-100	Total plants	Mean % infection of population
L. elongatum	2	8	1	0	0	0	0	0	0	11	3.75
L. elongatum (4x)	2	1	0	0	0	0	0	0	0	3	2.44
A. cristatum	0	0	0	0	0	0	0	0	2	2	88.33
Et. pycnantha	0	0	0	0	0	0	0	0	1	1	100.00
Et. elongatiformis	0	0	0	0	0	1	0	0	0	1	48.33
Th. nodosum	1	0	0	0	0	0	0	0	0	1	2.00
Ley. secalinus	1	0	0	0	0	0	0	0	0	1	0.00
Ley. Angustus	1	0	0	0	0	0	0	0	0	1	1.00

An AFLP Genetic Map of *Gibberella zeae*

Jurgenson, J.E., Bowden, R.L., and Leslie, J.F.

A DON-producing strain of *Gibberella zeae* isolated from wheat in Kansas was crossed with a NIV-producing strain isolated from barley in in Japan. Strains were marked with complementary nitrate nonutilizing (*nit*) mutations. Random ascospores were collected and planted on nitrate medium. Ninety-nine wild type recombinant progeny were collected and AFLP patterns were produced with 13 different primer pairs using two selective bases per primer. Four hundred and seventeen markers were scored. To date, 200 have been placed on the genetic map. Eight linkage groups were identified ranging from 391.5 cM to 8.5 cM in length. The total genetic distance for all linkage groups was 1057.8 cM. The median distance between markers in a linkage group was 3.2 cM. We intend to add more markers to the map to improve the resolution. This genetic map may be useful for cloning and for population genetic studies.

P

Resistance to Fusarium Head Blight in a Chinese bread wheat

H. Ma (1), R.H. Busch (2), and R. Dill-Macky (1).

This study investigated the genetic basis of resistance to Fusarium Head Blight (FHB) in a Chinese bread wheat in the field and greenhouse (GH), and the possible association of resistance with spike color (light green [LG] vs. dark green [DG]). A total of 110 $F_{5:7}$ random lines from the cross Wuhan (Resistance, LG) x Norm (Susceptible, DG) was evaluated at FHB nursery in St. Paul, MN in 1997. Disease index (DI) and spike color of each line were recorded. Disease severity (DS) was evaluated in the GH. The narrow-sense heretibility estimates were 71% (DI) and ~68% (DS). Rank correlations were 0.50 between field and GH readings, and 0.65 between successive GH readings. The resistance in Wuhan appeared to be controlled by 3 genes if only those lines that were classified as resistant in both field and GH were considered. No transgressive segregants were identified. The DI and DS mean of LG lines were consistently lower (indicating higher resistance) than the mean of DG lines. Therefore, the LG spike color may be a useful morphological trait for selection of FHB resistance.

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GERMPLASM INTRODUCTION AND EVALUATION IN WINTER, SPRING, AND DURUM WHEAT

Anne L. McKendry[1], Paul Murphy[2], Yue Jin3, and Elias Elias[4]

INTRODUCTION

Fusarium head blight, also known as scab is an increasingly important problem in the north-central region of the United States because of the emphasis on conservation tillage, the lack of effective cultural and/or fungicide control and the lack of effective sources of genetic resistance. In addition to reduced kernel density and color at harvest, associated deoxynivalinol (DON) accumulation in the grain prevents it from being marketed. Host resistance offers the most promising tool for scab management but breeding has been hindered by a lack of effective resistance genes. The discovery of new, genetically different sources of resistance is critical to the development of cultivars with effective levels of stable resistance. It is likely that different genes condition scab resistance observed in lines from different geographic regions, however, there has been no systematic search of the national collections for scab resistance genes, nor has there been an effective system for germplasm exchange with breeders in countries where scab is a major concern . There is, therefore, an urgent need to screen germplasm lines from different geographical regions in an effort to identify genetically different sources of resistance to this disease. In addition, there is a need to develop effective mechanisms whereby germplasm can be exchanged with cereal breeders internationally who have made strides in developing resistant germplasm. Finally, resistant germplasm and information on new sources of resistance needs to be disseminated to breeders nationally. Only then can the threat of scab to all producers, processors, and consumers of wheat and barley be minimized.

OBJECTIVES

Germplasm research conducted within the National Wheat and Barley Scab Initiative will be conducted in the four major commodities currently at risk including spring and winter wheat, durum wheat and barley. The overall objective of the germplasm research is to initiate an aggressive world-wide search for resistance to scab through: 1. a systematic search of wheat and barley cultivars, breeding lines and land-races currently maintained in the National Small Grains Collection; 2. the introduction of resistant germplasm through international contacts; and, 3. the development of an International Scab Germplasm Nurseries to facilitate the exchange of scab resistant germplasm globally. Following verification and/or purification of resistance, germplasm will be distributed to breeders in at risk states.

MATERIALS AND METHODS

The search for resistance will initially target those geographical areas where resistance has been identified, where environmental conditions are conducive to scab development, or where there are active scab resistance breeding programs. Germplasm will be introduced into the United States or acquired from the National Small Grains Collection and will be screened for scab resistance and important agronomic traits under artificial inoculation in the field and/or in the greenhouse. Resistance will be verified under greenhouse conditions and resistant lines will be distributed to breeders in at risk states. Over a five year period and in collaboration with other breeders, the deployment of both major and minor genes for scab resistance will be initiated through their introgression into adapted cultivars. Information on lines screened, resistance levels observed and associated agronomic traits will be incorporated into databases for both wheat and barley that will facilitate wide-spread dissemination of the information collected. Data may be made available through: the GRIN (Germplasm Resources Information Network), GrainGenes and/or the National Wheat and Barley Scab Initiative Web Site.

Research has been initiated in all four commodities in FY98 under the direction of the following area leaders.
Winter wheat: Dr. Anne L. McKendry, University of Missouri; Dr. Paul Murphy, North Carolina State University

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Spring wheat: Dr. Yue Jin, South Dakota State University
Durum: Dr. Elias Elias, North Dakota State University
Barley: Dr. Don Rasmusson, University of Minnesota

WINTER WHEAT GERMPLASM EVALUATION

Anne L. McKendry, Agronomy Department, University of Missouri, Columbia, MO

J. Paul Murphy, Department of Crop Science, North Carolina State University, Raleigh, NC

Winter wheat germplasm will be evaluated at two locations: the University of Missouri and North Carolina State University.

At the University of Missouri, 937 accessions from the USDA collection at Aberdeen, Idaho will be evaluated including accessions from China, South Korea, Japan, Brazil, and Italy. Seedlings will be planted in the greenhouse in the fall of 1998 and inoculated at first anthesis with 10mL of a 50,000 spore/mL suspension of *Fusarium graminearum* placed in a single central floret. Plants will be incubated in a mist chamber for 72 h post-inoculation to promote disease development. Ratings for Type II resistance will be made at 18 d after inoculation. At maturity, heads will be harvested and evaluated for kernel quality. During the fall of 1998, the same accessions will be planted as head rows in the field at Columbia, MO. Plants will be sprayed using a 50,000 spore/mL suspension at 75% heading, maintained under overhead mist irrigation through heading and evaluated for scab incidence and severity 18 d after inoculation. At maturity, a random sample of 20 heads will be harvested for kernel quality evaluation including number of tombstones, and kernel weight. Data on winter survival, maturity, height, septoria leaf blotch resistance, and other relevant agronomic traits will be collected in order to assess adaptation of lines evaluated.

At North Carolina State University, approximately 300 diverse germplasm accessions including synthetic hexaploids and accessions of *Triticum aestivum* (including some spelt accessions), *T. tauschii*, *T. cylindricum* and *T. monococcum* will be planted in the greenhouse in the fall of 1998. Resistance will be evaluated using single floret inoculation. Cultivated entries, will be planted in the field at Kinston, NC in a three- replicate field evaluation. Disease evaluation will be conducted using a conidial spray suspension at flowering under overhead mist irrigation. Inoculations will be spaced at 3-5 d intervals to coincide with variation in flowering dates. Two to three weeks after inoculation, data will be recorded for scab incidence and severity. Following harvest, a random sample of 1000 seeds will be examined and number of infected kernels recorded.

Data from both locations will be provided to breeders in the winter wheat region and will be made available at the 1999 Scab Forum.

DURUM WHEAT GERMPLASM EVALUATION

Elias M. Elias, Department of Plant Sciences, North Dakota State University, Fargo, ND

In previous studies prior to 1998 in collaboration with Dr. Robert Stack, Dept. of Plant Pathology, NDSU, several durum genotypes representing germplasm from North America and the Middle East were screened for Fusarium Head Blight (FHB). A variation in infection existed among these genotypes, few lines had a very moderate level of resistance (MR or less susceptible) to FHB. In addition to this germplasm, Langdon dicoccoides substitution lines LDN (DIC) developed by Joppa and Cantrell were crossed to the variety Vic and evaluated for FHB. Indications are that LDN (DIC-3A) has reduced infection to FHB. The level of resistance present in the above germplasm is not adequate to develop highly resistant durum cultivars.

The search for better sources of resistance is essential to insure the development of FHB resistant durum cultivars. The objective of the Durum Germplasm Introduction and Introgression Program (DGIIP) is to screen a wide range of durum germplasm including the world collection with FHB. In the summer of 1998 100 accessions were obtained from Dr. Harold Bockelman, National Small Grain Collection for FHB evaluations. The accessions were planted in two replicates as hill plots in the FHB screening nursery at Prosper, ND. In addition to the natural epidemic, in collaboration with Dr. R. Stack an artificial epidemic of *Fusarium graminearum* was created by spreading infected corn kernels on the ground. A good epidemic was created but disease ratings on these accessions were not possible because the accessions were not adapted to the North Dakota growing environment.

An additional 400 accessions were obtained from Dr. Harold Bockelman for FHB evaluation. A total of 500 accessions were sent to the Academy of Agricultural Sciences, Plant Protection Institute Shanghai, China to be evaluated for FHB in the 1998-99 growing season. Also 50 accessions were sent to Groupment Agricole

Essonnois (GAE) in France for evaluation.

We would like the DGIIP to be cost effective in future screening. We are exploring the possibility of large greenhouse screening nursery at NDSU. Since durum wheat grows very well in our winter nursery in New Zealand we will try to establish a permanent FHB screening nursery there.

SPRING WHEAT GERMPLASM EVALUATION

Yue Jin, Plant Science Department, South Dakota State University, Brookings, SD

Selected accessions of hexaploid wheat with spring habit in the National Small Grains Germplasm Research Facility (Aberdeen, ID) were evaluated for resistance to scab in a field nursery. A total of 425 accessions of hexaploid wheat with spring habit were selected from Brazil, eastern China, Italy, and Japan. Spring wheat cultivars Sonalika (susceptible, early), Wheaton (susceptible, late), Bacup (resistant, early), and line ND2710 (resistant, later) were used as checks. Check-to-entry ratio was 1 to 30. Entries were planted into one-meter rows. Individual entries were tagged when 75% (or more) plants in a row were at anthesis. Tagged rows were inoculated using a conidial inoculum at a rate of 50 mL/row with a suspension of 75,000 conidia/mL. A second inoculation was done one week later. In addition to spray inoculation, corn and oat kernels infected with *Fusarium graminearum* were spread weekly for three consecutive weeks, starting at the jointing stage of most entries. After inoculation, plots were mist-irrigated for five minutes in a 30-minute interval between 8:00pm and 8:00am. Notes on disease incidence and severity were taken 17 days after the first inoculation. A sample of 15 spikes was used to evaluate disease in each entry. When fewer than 15 spikes were available in a row, all spikes were evaluated. Disease index was derived by multiplying the average severity score with incidence. Two percent of accessions had a disease index lower than the resistant check ND2710, and 22% accessions lower than Bacup. Individual plant/spike selections were also made in some accessions. Further evaluations will be conducted to verify these putative sources of resistance.

Transferring Scab Resistance to Southern Soft Red Winter Wheat

E. A. Milus and C. T. Weight

Objective

To combine genes for scab resistance into lines adapted to the southern soft red winter region

Introduction

Currently, all wheat varieties adapted to the southern soft red winter wheat region are susceptible to scab. Severe scab epidemics occurred in this region in 1990 and 1991, but only sporadic, light infestations have occurred during the past seven years. Because epidemics are infrequent and unpredictable, scab resistance will need to be combined with acceptable levels of yield, test weight, quality, and resistance to other more prevalent diseases in order for scab-resistant varieties to be widely grown. This research is funded by the Arkansas Wheat Promotion Board.

Materials and Methods

Agripro Mason and Pioneer variety 2684 were chosen as adapted parents because their short vernalization requirements and photoperiod sensitivities provide wide adaptation in the southern soft red winter wheat region and facilitate producing more than one generation per year. Agronomically suitable CIMMYT spring wheat lines with scab resistance and resistance to other diseases such as leaf rust, stripe rust and Septoria tritici blotch were the primary sources of scab resistance. Other sources included Chinese, Croatian, and northern soft red winter wheats. Crosses were made in the greenhouse during the winter, always using the adapted parent as the female. Backcross, topcross, and F_2 populations were produced in growth chambers and greenhouses during the summer. The populations, adapted parents, and early- and late-maturing check varieties were space-planted in the field in October 1997 at the Vegetable Substation, Kibler, AR.

The populations were selected for traits other than scab resistance during the first field season. In order to initiate disease epidemics, spreader rows of a susceptible variety (Hart) were inoculated with a mixture of prevalent leaf rust races, and certain populations expected to segregate for stripe rust or Septoria tritici resistance were inoculated with local cultures of stripe rust and *Septoria tritici*, respectively. Populations were rogued several times to eliminate undesirable types. An unusually mild winter allowed spring wheats to survive so plants that headed before the early-maturing check variety were rogued to remove as many spring types as possible. Plants that were too tall, too short, too susceptible to leaf rust, stripe rust or Septoria tritici blotch, had low yield potential, or were later than the late-maturing check variety were also rogued. If a population had a preponderance of undesirable types, the population was eliminated rather than rogued.

Of the populations in the field, 117 F_3, backcross F_2, or topcross F_2 populations were selected for advancement over the summer. The optimum number of heads per selected population was 165. One head from each of 165 of the best plants was harvested if the number of good plants exceeded 165. If there were less than 83 good plants, then two heads per plant were harvested. If the number of good plants ranged from 83 to 165, then one head per plant was harvested and a second head was harvested from enough plants to obtain a total of 165. Heads were dried, and one or two seeds per head were germinated using a standard procedure to overcome seed dormancy. Seedlings were vernalized either in a vernalization chamber or a growth chamber, and plants were grown in a growth chamber for about two months before being transferred to a greenhouse. The advanced populations will be planted to the field in October 1998 as head rows. A mist system will be installed in the field, and plants will be inoculated with a mixture of *Fusarium graminearum* isolates in order to identify scab-resistant lines with resistance to other diseases as well as good agronomic type. Residual seed from the harvest at Kibler was bulked and will be planted at the Northeast Research and Extension Center at Keiser, AR, to determine the adaptation of the populations on a heavy clay soil.

Results and Discussion

In general, the CIMMYT lines combined well with the adapted parents, and the plant type and yield potential in many of the populations were exceptionally good. Plant type in the populations with Croatian or Chinese sources of resistance were not as good, but the best plants in the best populations were selected with the expectation that they may have different genes for resistance. The Chinese line, Wang-Shui-Bai, combined very poorly with whatever it was crossed to, and no plants were saved from populations with Wang-Shui-Bai in the pedigree.

The summer of 1998 was one of the hottest on record, and little or no seed was produced by many populations that were transferred from the growth chamber to the greenhouse in July. Populations that flowered in the growth chamber during September appear to have acceptable seed set. The generation to be planted to the field this fall will be determined on a population by population basis depending on the number of heads per population that produced seed during the summer.

Populations[1] being advanced to incorporate scab resistance into southern soft red winter wheat.

CIMMYT SOURCES

NG8675 / Catbird
P2684 / Mason
2 * P2684
Mason / Freedom
Mason
P2684
Freedom

Choix M95
Mason / P2684
Mason
P2684
P92823A1-1-2-3-5

Catbird [2]
P2684 / Mason
Mason
2 * Mason
Freedom

Sha 3 / Catbird
P2684 / Mason
Mason

Sha 3 / Kau2
2 * Mason

Sha 5 / Weaver
P2684 / Mason
Mason

Chil / Chum 18
P2684 / Mason

Alucan / YMI 6
2 * Mason

Chu 18 / Seri
Mason

Chil // Ald / Pvn
Mason

N7840 // Parula / Veery 6
P2684

(Gov / Az / Mus / 3 / Dodo / 4 / Bow
D48 x 42 x 6
P2684
P92823A1-1-2-3-5

Frontana
P2684 / Mason
Freedom

Chinese sources

Yan-Shi 9
P2684 / Mason
Mason
P2684
Freedom

N895004-1
P2684 / Mason
P2684

N894037-1
Mason / P2684

Er-Mai 9
Mason
P2684
Freedom

Yu-Mai 7
Mason
P2684
Star
Freedom

Croatian sources

Super Zlatna
Terrenzio
Freedom
Mason / Freedom

Bizel
P92823A1-1-2-3-5
Mason / P92823A1-1-2-3-5

Northern SRWW sources

P92823A1-1-2-3-5
Mason / Freedom
P2684 / Mason
Mason
P2684
Parula
Bow / Crow // Bau...
Parula // Mason
Freedom

Freedom
Mason
P2684
Bau / Milan
Fasan
Mango / Veery 10 / Parula
Pastor

[1] Populations are in the F_3/F_4, topcross F_2/F_3, or backcross F_2/F_3 depending on the cross and whether enough seed will be obtained from the summer greenhouse.
[2] Seven different Catbird lines were used and are combined here for simplicity.

Transformation Work at the University of Nebraska

Amit Mitra, M. Dickman, Tom Clemente, Shirley Sato, and P. Stephen Baenziger,

Below is a table summarizing three of our projects in wheat transformation: a) promoter analysis, b) disease resistance, and c) developing an *Agrobacterium tumefaciens*-mediated transformation system. The promoter data presented here is to study the effects of various constitutive promoters and effect of introns. The adenine methyl transferase gene promoter (*amt*) was discovered at the University of Nebraska and is patented by this institution. Numerous additional putative promoters are currently being studied. The disease resistance work is to develop novel disease resistance mechanisms. Lactoferrin, lactoferricin (the antimicrobial domain of the lactoferrin molecule), and oxalyl-CoA Decarboxylase are known to have anti-fungal properties and in some cases anti-viral properties. The 2-5A system if successfully transferred to wheat may provide resistance or tolerance to every known economic RNA virus of wheat. New monocot customized constructs with better promoters and using additional genes are being developed for future transformation experiments to develop disease resistance plants. While the two previous research areas use microprojectile bombardment, we are committed to developing an *A. tumefaciens*-mediated transformation system because it should provide more stable inserts and less copy number problems.

Construct	Description	Independent Events*	R1 Seed	R2 Seed
Promoter Analysis				
p35s	Core 35s	5 (14)	yes	Collecting
pUPUI	Rice Ubi-int	8 (8)	yes	Collecting
p35sU1	Core 35s-Ubi int	13 (16)	yes	Collecting
pMU1	Mas-Ubi int	4 (35)	yes	Collecting
pSPG	"Super promoter"	5 (19)	yes	Collecting
pSPU1	"Super promoter"-Ubi int.	24 (26)	yes	Collecting
pE7131	Modified 35s	9 (26)	yes	Collecting
pUbi	Rice ubi promoter	2 (7)	yes	Collecting
pAmt	Amt promoter	3 (10)	yes	Collecting
pMAS	mas promoter	2 (8)	yes	Collecting
pNos	Nos promoter	2 (13)	yes	Collecting
Disease Resistance				
p Oxalase	Oxalyl-CoA Decarboxylase	8 (12)	yes	To be planted
p Lactoferrin	Lactoferrin	3(11)	yes	To be planted
p Lactoferricin	Lactoferricin	In progress(8)	yes	To be planted
p 2-5A	2-5 system	9 (25)	yes	yes
Agrobacterium-mediated				
pPTN115	35snpt II +Mod 35s-Gus	11 (9 [5 N.T])	yes	collecting

* First number indicates total number of independent events either co-expressing GUS (promoter analysis and Agrobacterium sections) or presence of non-selected cassette by PCR and/or Southern blot analysis. Number in parenthesis represents total number of independent events that were positive for the selectable marker by ELISA. Agrobacterium section 5 independent events have not been tested [N.T.]

TRANSFORMATION OF BARLEY WITH ANTIFUNGAL GENES AND STRATEGIES FOR THEIR TARGETED EXPRESSION

[1]Ronald W. Skadsen, [2,3]Anna M. Nuutila, [2]P. Sathish, [2]Heidi K. Kaeppler

OBJECTIVE

To redirect the expression of genes encoding natural antifungal proteins so that they will provide barley and wheat with resistance to *Fusarium*.

INTRODUCTION

Cereal crops are challenged annually by fungi which destroy vast acreage of crops or render grain unfit for consumption. Losses of cereals to scab (Fusarium head blight or FHB) average hundreds of millions of dollars each year in the U. S. alone. Although there is no known barley or wheat germplasm with resistance to Fusarium, there are natural plant defenses which can potentially be enlisted against FHB. Plants marshal a number of defenses against infections by pathogens, pests and abiotic stresses [2]. Besides physical barriers and existing chemical defenses, various compounds may be synthesized in response to localized infections. The early "hypersensitive response" impedes the establishment of pathogens and leads to a chemical systemic acquired resistance [SAR; 13] which helps to protect against further infection. SAR is initiated in response to chemical signals from the infection sites by chemical messengers such as jasmonic acid [3] and salicylic acid [10]. Among the protective adaptations is the synthesis of pathogenesis-related proteins [PRPs; 9].

Antifungal proteins are synthesized in response to infections or are deposited in fruits and storage organs during normal development [17]. These are classified into five families of PRPs [1]. While the mechanism of action of PR-1 proteins is unknown, PR-2 proteins include b-(1,3)-glucanases, PR-3 proteins include chitinases, and PR-4 proteins bind to chitin of hyphal cell walls [6]. PR-5 proteins form a group which is comprised of osmotin [15], PRHv-1 [5], PWIR2 [11] and other thaumatin-like proteins (TLPs). A group of TLPs, permatins, has been found to be widespread in cereal seeds. Zeamatin was first purified from maize seeds [12], and its ability to permeate and lyse fungal hyphae suggested the term "permatins" [20]. Homologous proteins were found in seeds of barley (hordomatin) [7] and oat (avematin) [20; GenBank accession C33174]. The permatins are small proteins of 21-24 kD, and the ability of antizeamatin serum to cross-react with proteins of similar size from a wide range of cereals suggests that they are ubiquitous and structurally related [19]. Thionins, including the hordothionin storage protein of barley, belong to a class of small high-cysteine proteins. these have potent antimicrobial properties which have been known for decades [4].

Inoculation of oat with an incompatible isolate of the oat stem rust fungus (*Puccinia graminis* f. sp. *avenae*) results in the production of four TLPs, RASTL1-4 [8]. Also, the inoculation of wheat with non-pathogenic barley powdery mildew fungus (*Erisiphe graminis* f. sp. hordein) increases resistance to subsequent inoculations with pathogenic wheat powdery mildew fungus (*E. graminis* f. sp. *tritici*) [11]. This SAR response induces at least six putative defense genes, one of which encodes a TLP.

Despite the fact that thionins, permatins, TLPs and other PRPs have antifungal properties, recurring large-scale fungal infections demonstrate that these proteins, as they are currently expressed, cannot fully protect cereal crops. However, knowledge of their expression and mechanism of action could lead to more effective antifungal strategies through biotechnology approaches. Our approach in developing Fusarium-resistant lines of barley is to clone genes which already encode potential anti-Fusarium proteins in the barley seed. If the transgene products are consumed, there should be little problem with product acceptance, since they are regularly consumed anyway. At present, the genes will be expressed constitutively, as a model system. Later, we hope to develope tissue specific promoters. Current research in ours and other labs will identify the best targets for the expression of these genes.

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MATERIALS AND METHODS

To obtain developing seeds, Morex barley and Prairie oat were grown in a greenhouse maintained at 18 to 27°C and received supplemental lighting. RNA from oat leaves infected with stem rust fungus (*P.* f. sp. *avenae*) was generously provided by Bill Bushnell, Cereal Rust Laboratory, U.S.D.A., St. Paul. RNA samples were from leaves harvested 36, 48 and 60 h after inoculation [8]. Barley spikes infected with a suspension of *Fusarium graminearum* were a gift from Brian Steffenson, North Dakota State University, Fargo. Spikes of the Chevron and Steptoe cultivars were inoculated when seeds were in the milky to mid-doughy stages and were left in a mist chamber for 27 h. Control spikes were misted with water. Spikes were frozen in liquid nitrogen, and seeds were dissected from the frozen spikes. RNA was extracted from these seeds and from developing seeds and vegetative tissues. Seeds of barley and oat were sampled from the fertilization period to the mature and germinated stages.

The permatin clones pOATPERM1 (GenBank U57787) and pBARPERM2 (AF016328), the B3 hordein clone pHORDB3 (AF016237 and AF016238), the barley actin clone pACT10-4 (U21907), and the barley clone homologue to the cell division cycle protein CDC48 (pBCDC48, AF045927) were obtained by probing our cDNA libraries made from pooled mRNA of seeds in the early, middle and late stages of development. Plaque lifts were probed with ^{32}P-labeled mixed oligonucleotides corresponding to the conserved amino acid sequences of TLPs. The barley permatin clone pBARPERM1 (GenBank AF016327) was obtained from a PCR in which an aliquot of the ss-cDNA from the barley library construction was used as a template. The 3' primer corresponded to the last seven amino acids of the deduced coding sequence of pBARPERM2, and the 5' primer corresponded to the sequence encoding the first seven hordomatin amino acids, previously determined for the processed protein [7]. The oat seed permatin clone, pOATPERM1 (U57787) was isolated from a cDNA library, constructed as above, from pooled mRNAs representing the early, middle and late stages of development of Prairie oat seeds; the clone was detected by probing the library with the TLP oligonucleotides, as above. For probe synthesis, insert DNAs were labeled with ^{32}P-dCTP. RNA blot and Southern blot analyses, radiolabeling and genomic DNA purification were conducted exactly as previously described [16]. Clones were sequenced by dideoxy chain termination [14].

For genetic transformation of barley and oat, expression vectors were constructed from the pAHC25 ubi/GUS/BAR cereal expression vector, provided by Peter Quail. In these constructs, the GUS gene was removed and replaced with either pOatperm1, a hordothionin cDNA clone, or pBarperm1 (in the reversed or antisense orientation). Barley (Golden Promise) transformation was conducted by A.M.N., and oat transformation was conducted by H.F.K. using particle bombardment. The pBarperm1 coding sequence was cloned into the pET28 bacterial expression plasmid, and the resulting permatin protein was purified by nickel and CMC chromatography (by P.S.). The N-terminus was sequenced, and the confirmed protein is currently being used to produce antibodies.

Antifungal testing will be conducted with the aid of a strain of *F. graminearum* which produces a bright green fluorescence when illuminated with short-wave blue light. This strain was created by Tom Hohn (USDA, Peoria) by genetic transformation with the green fluorescent protein gene of jellyfish. Details of this procedure will be published elsewhere. Antifungal testing will also be conducted by inoculating Fusarium (non-transformed) spores in dilute carrot juice media in large-well microtitre plates, supplemented with plant extracts or purified proteins.

RESULTS AND DISCUSSION

Antifungal thaumatin-like proteins (TLPs) of seeds, known as permatins, occur in many cereal seeds, but little is known of their regulation and roles. Permatin cDNA clones, pBARPERM1 and 2, were produced from developing barley seeds, and pOATPERM1 was produced from oat. Sequence comparisons suggested that deletions in specific elements were involved in the evolution of the (smaller) leaf TLPs. Developing barley and oat seeds accumulate permatin (and oat globulin) mRNA in an unusual bimodal pattern. Peak mRNA levels occur very early, followed by sudden decreases to near-zero levels and a second peak in the doughy stage. Barperm1 mRNA is largely confined to tissues surrounding the starchy endosperm, while Oatperm1 mRNA is more evenly distributed between the endosperm and surrounding tissues. Small amounts of permatin mRNAs also occur in roots and epicotyls. Although oat leaves infected with *Puccinia graminis* were previously found to produce high levels of the leaf-specific TLP, RASTL1, only modest levels of Oatperm1 mRNA (normally not found in healthy leaves) were produced. Developing seeds of Chevron barley, a cultivar moderately resistant to head scab (caused by *Fusarium graminearum*), had more Barperm1

mRNA than seeds of the highly susceptible cultivar, Steptoe. These levels increased slightly after inoculation with *Fusarium*, while other mRNAs sharply declined.

Transformation of barley with Oatperm1 was difficult and yielded only one regenerated plant. This plant eventually produced over 1700 spikes, most of which contained little or no seed. Only 1 to 5 seeds were produced on each of the 595 fertile spikes. A population of these is now growing in the greenhouse. These have the Oatperm1 gene present, as judged by PCR tests and Southern blots. Antibodies should soon be available from our purified Barperm1 protein, and these should cross-react sufficiently so that the level of permatin protein expression can be measured. The logic for transforming barley with the oat permatin gene was to reduce the chances of reductions in overall permatin expression due to co-suppression. The progeny have a distinctive phenotype which includes pronounced anthocyanin production and shortened middle internodes. Transformation of barley with pBarperm1 in the antisense orientation was undertaken in an effort to knock out the expression of the native gene. This was also successful and has produced a number of regenerants with a grassy phenotype, apparently affecting a basic process.

Transformation with hordothionin has been more successful, resulting in 100 healthy regenerants. Spikes are now developing on these plants. These also have a distinctive phenotype, which includes curly flag leaves and a slight wrinkled pattern on the surface of other leaves. Hordotionin levels in these plants will be measured immunologically by Berne Jones (USDA, Madison).

The Kaeppler lab has successfully transformed oat with pOatperm1 and is currently selecting cultures transformed with hordothionin (potentially the first oat expressing a thionin). These will be used in an attempt to produce rust-resistant oats.

When spikes from transformant progeny develope, preliminary screening for Fusarium resistance will be conducted with green fluorescent Fusarium infections. In preliminary tests, infections could easily be detected, since it is possible to see each spore and each mycelial thread. Tests with purified hordothionin showed that concentrations between 2.6 and 8.0 mg/ml completely kill *Fusarium* in solution.

We have recently begun using the differential display technique in order to detect genes which are expressed in spike tissues and not in flag leaves. The resultant DNA products will be used as probes to locate the corresponding nuclear genes, and from these, the promoter DNA sequences will be isolated. These will hopefully serve in the future as tissue-specific promoters to direct the synthesis of antifungal proteins in the tissues surrounding the seed, thus intercepting *Fusarium*.

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FUSARIUM HEAD BLIGHT (MYCOTOXINS, *FUSARIUM* SPECIES, DAMAGED KERNELS, SEVERITY) IN LOCAL AND INTRODUCED BARLEYS

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OBJECTIVES

To assess and compare Fusarium head blight in local and introduced (resistant) barleys.

INTRODUCTION

Since 1993, Fusarium head blight (FHB) has been detected annually in barley grown in Manitoba, Canada (3). Prior to this, and since 1986, FHB had been important only in wheat. In 1997, FHB was found in most fields of barley surveyed in southern Manitoba at incidence levels as high as 75% (average 31%) and severity levels of up to 40% (average 12%) (2). Fusarium head blight, caused by several *Fusarium* spp., results in yield loss and deoxynivalenol (DON) contamination, reducing the feed and malting value of barley grain. In wheat, visual severity and other parameters used to quantify the disease are correlated (1,4). The situation in barley is less clear, and determining the relationships among disease severity (visual FHB Index), *Fusarium* seed infestation, Fusarium damaged kernels (FDK) and DON in barley would help in understanding the disease. Until cultivars with superior resistance become widely available, producers will have to manage FHB in other ways. A useful component of such an integrated management approach is cultivar selection, i.e., planting cultivar(s) that are less susceptible to the disease. This study compares 16 local and 16 putatively resistant barley accessions for reaction to FHB, and examines correlations among the disease parameters outlined above.

MATERIALS AND METHODS

Sixteen representative spring barley cultivars recommended for production in Manitoba (Table 1) and 16 accessions reported to have resistance to *Fusarium* (Chevron, Fuji Nijo, Gobernadora, Harbin, Korsbyg, Krasnojarskij 1, Maris Mink, Murasaki-mochi, Nepolegajuscij, Seijo II, AC Sterling, Svanhals, Symko, Ussurijskij 8, Zaoshu 3 and Zhedar 1) were tested for reaction to FHB under natural conditions. The experiment was grown at Carman MB, as 4 m long, 4-row plots with two replications for adapted cvs., or single 1.5 m rows (due to limited seed) for accessions. Two weeks before maturity, 40 heads were collected at random per plot and stored at -20C to evaluate disease severity, based on the 'FHB Index': % incidence x %severity / 100. At maturity, the 2 centre rows (local) or entire row (accessions) were harvested to determine % *Fusarium* infestation (100 kernels), % FDK (10 g grain), and ppm of DON (20g ground sample, tested by ELISA). LSD values for measured parameters and correlation coefficients between them were calculated using SAS programs.

RESULTS AND DISCUSSION

The FHB Index for the 16 local cultivars ranged from 1.9 to 12.8% (Table 1). Among the accessions, 10 had an FHB Index <1%, but two, Fuji Nino (28%) and Korsbyg (37%) had the highest. *Fusarium* spp. infestation of kernels (8 - 68% in local, 3 - 46% in accessions) was considerably higher than expected from visual symptoms. *Fusarium graminearum* was the principal *Fusarium* species isolated from kernels; *F. equiseti* and *F. sporotrichioides* also were found. The high FHB Index for cvs. CDC Guardian and Manley may have been due, in part, to their very high levels of *C. sativus* seed infestation; this can discolor heads and produce symptoms on grain (brown staining of kernels) similar to those of FHB. DON levels ranged from 1.3 to 14.2 ppm in local, and <0.1 to 1.9 ppm in the barley accessions. Correlation coefficients in both groups were significant for DON vs. total *Fusarium* spp. or *F. graminearum,* and total *Fusarium* spp. vs. *F. graminearum* (Table 2). In the barley accessions, FHB Index was additionally correlated with DON and *F. graminearum*. If CDC Guardian and Manley are removed from the analysis for local cultivars, then FHB Index becomes significantly correlated with DON, *F. graminearum* and *Fusarium* spp. (data not shown). Some of the differences between the local barleys and the accessions may have resulted from micro-environment effects due to plot size. For purposes of breeding for resistance, a simple measurement, such as visual severity, is desirable for assessing FHB reaction. This is valid for wheat (1,4), but further investigation will be needed in barley to verify this. The local, adapted cv. AC Oxbow

had low levels of DON, *Fusarium* and FDK, as well as a low FHB Index, making it a candidate for an integrated approach to FHB management. This and several other barleys tested should be useful as parents in breeding for improved FHB resistance in Canadian barley. *Cochliobolus sativus* may be suppressing FHB and/or *Fusarium* recovery.

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Table 1. Reaction of barleys to Fusarium head blight at Carman Manitoba in 1997.

Cultivar	Type[a]	DON ppm	*F.* spp. %	*F. gram.* %	*C. sativus* %	FDK %	FHB Index[b] %
Falcon	6r, hl, f	1.3	21.5	6.0	11.0	0.4	2.9
CDC Guardian	2r, f	1.8	8.0	2.5	90.5	0.8	12.4
AC Oxbow	2r, m	2.0	27.5	13.0	32.0	3.4	3.3
AC Metcalfe	2r, m	3.4	35.5	15.0	34.0	2.0	3.4
Manley	2r, m	3.8	25.0	2.5	62.0	1.0	12.8
Bridge	2r, f	4.1	35.5	24.0	32.0	4.1	4.0
Duke	6r, f	5.1	64.5	52.0	8.5	2.3	6.1
Tankard	2r, m	5.2	56.0	28.5	17.5	2.2	4.9
AC Lacombe	6r, f	5.5	50.5	23.0	13.0	2.9	9.4
CDC Earl	6r, f	6.6	61.0	39.5	20.5	1.9	12.0
Bedford	6r, f	6.7	58.0	37.5	9.5	3.3	7.5
CDC Silky	6r, hl, f	6.8	28.5	18.5	6.0	1.8	1.9
Heartland	6r, f	7.2	62.5	51.5	22.0	3.1	9.7
Stander	6r, m	10.1	67.0	46.0	13.5	2.7	7.1
Argyle	6r, m	10.4	54.0	41.5	6.0	1.5	5.2
AC Rosser	6r, f	14.2	67.5	45.5	11.5	5.1	11.1
LSD (0.05)		*7.2*	*21.6*	*17.6*	*12.4*	*2.5*	*3.4*
Mean (local)		5.9	45.2	27.9	24.3	2.4	7.1
Mean (accessions)[c]		0.6	16.3	7.0	25.4	1.0	4.8

[a] 2r=two-rowed; 6r=six-rowed; hl=hulless; f=feed quality; m=malting quality
[b] FHB Index = % incidence x % severity / 100
[c] individual data not shown

Table 2. Correlation coefficients for FHB in barley: local (top right); accessions (bottom left)

	DON	FHBI	*F.* spp.	*F. gram*	FDK
DON		0.21	0.77***	0.75***	0.25
FHBI	0.63**		0.25	0.14	0.38
*F.*spp.	0.75***	0.39		0.94***	0.20
F. g.	0.93***	0.69**	0.81***		0.20
FDK	-0.04	0.27	-0.07	0.02	

*,**,*** significant at P=0.05, 0.01 or 0.001, respectively
FHBI = FHB Index (% incidence x % severity / 100)

Evaluation Of Wheat Cultivars And Breeding Lines For Resistance To Fusarium Head Blight In Kentucky.

D. A. Van Sanford, B. Zeng, and C. S. Swanson, and B. Kennedy

OBJECTIVES:

1. To identify resistance to FHB in adapted genotypes.
2. To develop field-based protocols for resistance screening that can be incorporated into a breeding program.

INTRODUCTION

Since 1991, *Fusarium* head blight, or head scab, has caused significant losses in the SRW wheat (*Triticum aestivum* L.) crop in Kentucky and much of the eastern wheat region.

Conservation tillage has been cited as a major reason for the increased incidence of head scab in the SRW wheat crop. In Kentucky, virtually all of the wheat is planted after corn, following minimal or no tillage. If FHB levels are related to corn residue, scab will likely continue to be a problem in Kentucky and states in which the wheat-soybean-corn doublecropping rotation is prevalent. For these reasons it is imperative that we identify resistance genes that can be deployed in new varieties.

MATERIALS AND METHODS

Entries in the 1998 Uniform Winter Scab Nursery along with a number of advanced breeding lines (Tables 1, 2) were planted in a randomized complete block design with three replications. Experimental units were small plots (20 ft^2) with 6 rows planted in 7" rows using a Hege headrow planter. The planting date was 21 Oct. 1997. The previous crop was corn (*Zea mays* L.) and the seedbed had been chisel plowed and disked.

Our inoculation procedure was modeled after the method of Paulitz (1996) with modifications suggested by Lipps (Pat Lipps, personal communication, 1997). An isolate of *F. graminearum* was obtained from scabby wheat seeds, by seed surface sterilization and plating on acidified potato dextrose agar (APDA). Mycelium from this culture was then transferred to carnation leaf agar (CLA) to induce sporulation. A pure culture was obtained by single-spore isolation and cultures were increased on PDA. Mason jars containing 500 g of autoclaved corn (*Zea mays* L.) seed were inoculated with 6 mycelial plugs from the single-spore culture. Inoculated corn was maintained at room temperature for approximately 3 weeks; jars were shaken occasionally to help disperse inoculum within each jar. About 10 days before heading, we spread approximately 225 g of corn/plot among the wheat rows. Just prior to anthesis, mist irrigation of the plots was initiated for approximately 1 hour morning and evening throughout anthesis into early grain fill.

Evaluation of scab symptoms was done at approximately 19 DAA. Incidence of scab in 4 row-feet (2 2' rows) was estimated by recording the number of heads showing typical scab symptoms. Severity was estimated by counting scabby florets on 5 random heads per plot. The 2 2' rows were harvested with a sickle, total head number was counted, and grain was threshed in a single head thresher to estimate % scabby seed. Remaining plots were harvested with a Hege plot combine to measure grain yield and test weight.

RESULTS AND DISCUSSION

Our initial plan was to grow about 2 replications in another field for evaluation of Type II resistance (Mesterhazy, 1995) by injecting single florets. However we have had little success in adapting this method to field conditions in 1997 or 1998. One bonus from growing this material away from the irrigated study is that it gave us a control replicate to use as a baseline level of natural infection with which we could compare our inoculated plots (Table 3). One of the questions that must be answered is whether the level of scab that is achieved in irrigated, inoculated plots is much greater than is likely to occur in a natural infestation. If so, even slightly promising levels of resistance will be obscured. This concern is underscored by the low correlation (r=0.22) between inoculated and control plots, based on the data in Table 3.

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Table 1. Results Of The 1998 Winter Uniform Scab Nursery At Lexington, KY

Entry	Incidence	Severity	Index	Scabby Seed	Yield	Test Wt.
	% heads	% florets		%	bu/a	lb/bu
Patterson	72.6	90	65.34	25.14	38.1	44.8
Freedom	58.4	30	17.52	32.84	40.0	44.7
P2545	68	60	40.8	50.54	36.1	42.5
Emie	69.5	70	48.65	34.25	34.2	46.3
M94-1048	63.4	50	31.7	26.1	42.5	49.1
OH618	31.2	10	3.12	14.55	43.7	49.0
OH552	52.7	15	7.905	18.79	48.0	50.3
OH536	67.6	12	8.112	30.4	36.0	46.9
OH544	49.9	15	7.485	19.08	34.4	47.6
Wakefield	72.6	50	36.3	21.29	30.4	45.6
VA96-54-216	77.6	90	69.84	45.72	26.2	46.2
VA93-54-429	73.1	45	32.895	19.05	30.4	51.3
VA96-54-234	77.5	50	38.75	42.64	34.5	45.9
IL94-1909	55.2	15	8.28	19.73	46.1	51.2
IL94-1549	52.9	12	6.348	36	27.3	48.9
92823A1-1-4-4-5	45.3	45	20.385	17.8	39.2	47.2
92807A1-1-5-1-1	84.4	*	*	24.4	40.9	46.7
89118RC1-X-9-3-3	59.8	12	7.176	12.92	37.3	51.7
86958RC4-2-1-10	72.4	75	54.3	30.04	35.7	47.0
88288C1-6-2-8	70.9	35	24.815	25.85	33.1	47.1
92829A1-1-1-3-3	30.1	30	9.03	8.74	36.5	48.1
KS85W663-11-6-42	45.1	15	6.765	13.9	31.6	50.9
Geneva	65.3	57	37.221	34.05	21.7	43.2
Cayuga	56.6	17	9.622	20.09	19.7	45.5
NY85019-7117	56.1	35	19.635	36.73	27.7	43.4
NY87048W-7387	37.9	30	11.37	17.17	18.2	46.9
NY87047W-7405	79.9	90	71.91	27.63	24.9	42.9
NY64/H//H-7133	48.9	35	17.115	24.46	32.9	44.3
Agripro Foster	61.3	57	34.941	22.96	22.4	45.6
D5330	53.7	12	6.444	18.73	26.7	43.8
D4045	36.3	56	20.328	40.96	31.0	42.9
DC005	44	15	6.6	21.14	29.7	46.6
Ramrod	52.1	50	26.05	22.21	36.0	43.1
Mean	58.9	40.0	24.4	25.9	33.12	46.6
CV (%)	28.5	30.4	45.1	12.6	19.0	3.9
LSD (0.05)	29.4	20.6	19.7	30	9.8	3.6

Table 2. Evaluation Of SRW Wheat Breeding Lines For Scab Resistance, Lexington, KY, 1998.

Entry	Incidence	Severity	Index	Scabby Seed	Yield	Test Wt.
	% heads	% florets		%	bu/a	lb/bu
KY89C-895-14	36.41	23	8.37	20.1	29.3	47.9
KY89C-225-6	49.90	30	14.97	9.1	32.5	48.6
KY89C-888-29	51.60	30	15.48	40.6	25.4	43.8
KY89C-888-32	53.44	23	12.29	36.0	34.4	45.8
KY89C-744-19	54.06	23	12.43	42.9	37.2	46.2
KY89C-744-44	57.93	20	11.59	28.1	32.4	45.8
KY89C-225-11	58.61	40	23.44	27.3	31.1	45.8
KY89C-753-48	60.24	40	24.09	62.2	28.7	43.8
KY89C-744-57	61.34	33	20.24	46.8	33.8	45.0
KY89C-804-37	62.10	36	22.36	37.5	32.6	46.1
KY89C-007-3	62.87	30	18.86	24.6	37.2	48.0
KY89C-888-14	66.84	40	26.74	51.0	22.8	44.1
KY89C-225-5	67.28	30	20.18	44.1	37.4	45.8
KY89C-804-14	67.33	43	28.95	56.4	25.8	44.1
KY89C-752-21	71.86	26	18.68	57.1	31.6	43.2
KY89C-804-18	72.96	46	33.56	32.1	21.7	43.3
KY89C-873-43	72.97	30	21.89	42.9	23.5	49.1
KY89C-873-39	72.99	30	21.90	36.3	19.8	48.0
KY89C-720-10	73.12	46	33.63	53.3	26.4	42.3
KY89C-744-40	75.14	30	22.54	49.7	35.8	45.6
KY89C-804-23	75.65	40	30.26	61.7	28.1	43.1
KY89C-804-55	75.86	50	37.93	56.4	33.1	37.0
KY89C-804-11	84.48	33	27.88	47.4	26.9	46.6
Mean	64.56	33.57	22.10	41.90	29.89	45.18
CV (%)	26.2	28.6	39.1	26.8	19.9	3.4
LSD (0.05)	27.32	25.8	23.7	17.0	9.4	3.1

Table 3. Uniform Winter Scab Nursery Under Treated And Control Conditions, Lexington, KY, 1998.

ENTRY	Treated†	Control	Difference
	Scabby Kernels	Scabby Kernels	Scabby Kernels
	%	%	%
Patterson	25.1	10.1	15.0
Freedom	32.8	14.5	18.3
P2545	50.5	32.5	18.0
Ernie	34.2	19.2	15.0
M94-1048	26.1	4.0	22.1
OH618	14.5	18.1	-3.5
OH552	18.8	9.9	8.9
OH536	30.4	12.6	17.8
OH544	19.1	28.1	-9.0
Wakefield	21.3	11.8	9.5
VA96-54-216	45.7	9.9	35.8
Roane	19.0	18.8	0.2
VA96-54-234	42.6	24.3	18.3
IL94-1909	19.7	3.9	15.8
IL94-1549	36.0	4.8	31.2
92823A1-1-4-4-5	17.8	20.8	-3.0
92807A1-1-5-1-1	24.4	21.5	2.9
89118RC1-X-9-3-3	12.9	6.4	6.5
86958RC4-2-1-10	30.0	14.3	15.7
88288C1-6-2-8	25.8	29.2	-3.3
92829A1-1-1-3-3	8.74	6.6	2.1
KS85W663-11-6-42	13.9	7.2	6.7
Geneva	34.0	18.5	15.5
Cayuga	20.0	46.7	-26.6
NY85019-7117	36.7	12.2	24.5
NY87048W-7387	17.2	1.37	15.8
NY87047W-7405	27.6	10.0	17.6
NY64/H//H-7133	24.4	19.5	4.9
Agripro Foster	22.9	5.5	17.4
D5330	18.7	4.8	13.9
D4045	40.9	21.5	19.4
DC005	21.1	27.9	-6.7
Ramrod	22.2	22.4	-0.2
MEAN	25.9	15.7	10.2

† Inoculated with scabby corn and mist irrigated.

Transformation is the direct insertion of new genes into plants. Using transformation techniques, we can insert genes into barley and wheat that can't be incorporated by traditional crossing, such as genes from other plant species, animals, bacteria, or fungi. These methods also let us change the expression patterns of genes that are naturally found in wheat and barley. For example, there may be genes for proteins that inhibit fusarium growth that are turned on only in leaves. With transformation, we can alter these genes so they are turned on in the spike tissues that fusarium attacks.

Transformation of wheat and barley has the potential to help in the fight against FHB, in reducing both disease and toxin levels. But, these techniques are relatively new to wheat and barley, and there still are several areas that need improvement. This report will discuss some of these areas and will briefly describe some of the current barley transformation work at the USDA, ARS barley genetics lab in Fargo.

Barley and wheat transformation techniques currently require the use of tissue culture systems, in which cells are grown as unorganized clumps called callus, on artificial media. These calli are then induced to form green plants by altering the hormone levels in the media and placing them in the light. Plant regeneration from these calli is one of the first limitations in the system. Current transformation programs use the cultivars 'Bobwhite' in wheat and 'Golden Promise' in barley because they regenerate many plants. Neither of these cultivars meet current agronomic and quality standards. Using these older cultivars means more work to breed the new genes into modern cultivars. The newer barley and wheat cultivars generally do not regenerate many plants using standard tissue culture systems. This may soon change, as altered culture systems and artificial media compositions are being developed to improve regeneration rates from newer cultivars and advanced breeding lines.

Another limitation in transformation is the efficiency. The usual targets for transformation are immature embryos. The embryos are induced to form calli from which the transformed cells are selected. In typical experiments, only four to eight percent of these embryo-derived calli regenerate transformed plants. This low efficiency means time and resources are wasted. Research projects investigating ways to improve transformation efficiency are underway in both wheat and barley.

A third area that causes problems in transformation is somaclonal variation, the mutations caused by tissue culture. Most regenerated plants have lower yields than the cultivar they are derived from, and many have other undesirable mutations affecting traits such as heading data, height, seed weight, etc. Again, research projects are underway to reduce somaclonal variation.

Additional areas of research being investigated include gene stability and gene expression. Sometimes, an inserted gene that is expressed in the original regenerated transgenic plant is not reliably transmitted to its progeny. In other cases, the inserted gene is transmitted to progeny but the gene is inactivated and does not make its protein product. Altering the DNA code that makes up the gene, or adding sequences to the gene to target where it inserts into the chromosomes, can help overcome these problems. Other issues important in transformation include the genes used and the promoters that control expression of the genes. Dr. Pat Okubara will be discussing these areas in more detail.

I'd like to finish by providing a short summary of some transformation research going on in my barley genetics lab in Fargo. We are currently working with two genes, TRIr and PDR5, which may help reduce DON levels in FHB-infected plants. We have inserted these genes into Golden Promise barley and are currently testing the regenerated plants and their progeny for the presence and expression of the new genes. We will then be selecting the best transgenic plants for FHB and DON testing in the greenhouse and field. These lines also will be crossed to advanced lines from the FHB resistance breeding programs, to get the transgenes into adapted, high quality barley lines. We also have been investigating plant regeneration from current cultivars and advanced breeding lines using improved tissue culture systems and media. Several lines have been selected for use in future transformation efforts because they appear to have sufficient green plant regeneration from tissue cultures. Any of these lines would be an improvement over Golden Promise from a plant breeding perspective, and use of these lines for transformation will help speed the development of adapted, high quality, transgenic barley.