PREFACE

Fusarium Head Blight emerged in the past decade as a widespread and powerful enemy of American agriculture. This disease, also known as 'Scab', inflicts yield and quality losses on farms in at least 18 states. Food industries throughout the U.S. incur losses from the cost of dealing with the toxin-contaminated grain that often accompanies scab infection. Combined losses to all steps in the food system are difficult to estimate, but the bill at the farm-gate alone is estimated to exceed 3.0 billion dollars since 1990.

The existing private, state, and federal research system of the U.S. handles most of the newly emergent pests and diseases. But some problems, like scab of wheat and barley, present unusual challenges that warrant new approaches to research. There are several reasons why scab falls into this category:

- Scab is an economic threat to growers, processors and consumers of wheat and barley throughout much of the U.S.;
- Research is needed in a wide array of disciplines, with possible solutions including:
  - resistant varieties (from conventional or biotechnology approaches),
  - alternative residue and rotation management schemes,
  - crop protection via chemical and biological controls, and
  - detoxification or alternative processing of contaminated grain;
- The interactions of the scab pathogen with local and regional farming practices are complex and poorly understood;
- Recent experience clearly signals that no single institution can bring to bear the critical mass of research this problem demands;
- Scab solutions are likely to be both site and system specific, which dictates involvement of local experts in all of the directly affected states;
- Opportunities for acquiring competitive funds for scab research from traditional federal sources are extremely scarce.

As a result of these special circumstances, scab of wheat and barley is one of several plant disease complexes accorded special status as "Emerging Diseases" in recent Federal budgets. National awareness of the serious nature of scab is a welcomed development. However, the real challenge comes in the design and implementation of a national, multi-disciplinary, and multi-institutional research system that can win the war on scab as quickly and efficiently as possible.

During the past three years, federal, state, and private sector scientists have worked closely with growers, input providers, millers, and food processors from across the country to design and fund just such a system. The result of these efforts is the U.S. Wheat and Barley Scab Initiative. In one sense, the Initiative is a self-organized "contact group" on scab. The goal of the U.S. Wheat and Barley Scab Initiative is to develop as quickly as possible effective control measures that minimize the threat of Fusarium head blight (scab) to the producers, processors, and consumers of wheat and barley. The Initiative is guided by a Steering Committee that includes growers, farm organizations, food processors (e.g., millers, bakers, pasta manufacturers, and brewers), scientists (from Land Grant universities, USDA, and private companies), and consumer groups. Eight members of the Steering Committee also serve on an Executive Committee. The Steering and Executive Committees are advised by a series of six research committees composed of recruited volunteers from the scientific leaders of the U.S. wheat and barley research communities. The composition

of all committees is designed to ensure balanced input from all commodities, regions, disciplines, and institutions engaged in the battle against scab. The Initiative's Networking and Facilitation Office, based at Michigan State University, facilitates the work of these committees. That office also promotes communication among the varied parties interested in scab, and represents the Initiative's only 'fixed' asset.

Each year, the Steering Committee submits to the USDA-ARS a comprehensive and optimized research plan designed to achieve the Initiative's goals. That plan is the Initiative's recommendation for how the USDA-ARS can most effectively employ the funds appropriated by the U.S. Congress for collaborative scab research. The relevance of the research plan is assured by the central role that industry (growers and processors) plays in the Initiative's work. Much of the work reported in this document were partially or completed funded with Initiative recommended ARS funding.

Congress has appropriated funds for this collaborative effort for three consecutive years (FY98: $500,000, FY99: $3.5M, and FY2000: $4.8M). The USDA also contributed funds to Initiative recommended funding with end-of-year funds in FY97 ($200,000), and discretionary funds in FY98 ($200,000). The $4.8M appropriated in the FY99 budget is part of ARS's base budget and does not require the yearly lobbying process associated with 'special grants' through the USDA- CSREES. In other words, there is every reason to expect re-appropriation of the $4.8 M on an annual basis for at least several years. Increases specifically associated with scab have also been made in the base budgets of specific ARS facilities in the past three years ($1.375M). Combined, these funds represent an unprecedented commitment to wheat and barley research at the Federal level.

The FY97 and FY98 funds were dispersed primarily as Cooperative Agreements through Dr. Bob Busch's offices (USDA-ARS, MN). The Scab Initiative's Research and Steering Committees undertook a process in 1998 that generated a comprehensive research plan for the funds appropriated in FY99 federal budget (October 1998 through September 2000). Every conceivable solution area was considered, and the resulting mix of research projects is truly comprehensive. The prioritization process was competitive by its very nature, since researchers had to demonstrate their ability to contribute to the overall solution. On the other hand, the final research agenda consists of a mix of projects identified via both directed and purely competitive processes. In almost all cases, funds proposed by the Initiative will be heavily leveraged by existing sources of investments in personnel, facilities, and supplies.

The resulting research plan for FY99 involved 66 principal investigators in 19 Land Grant Universities and the ARS. Collectively, 111 projects were funded for one year, most with starting dates (dates funds were available) in May or June of 1999. The details of the overall research plan were finalized at the 1998 Fusarium Head Blight Conference. Project details including end of fiscal year (September) progress reports can be viewed at the Initiative's website at WWW.SCABUSA.ORG . Much of the work reported in these proceedings was partially funded by Initiative recommended funding.

The unprecedented nature, magnitude, and scope of this collaboration required constant innovation by the administrative staffs of the Initiative, ARS, and the participating Land Grant Universities. We are happy to report that the ARS has been supremely cooperative and has exhibited degrees of flexibility and sensibility not commonly attributed to Federal agencies.

The Initiative recently solicited proposals for fiscal year 2000. For the first time, a formal call for proposals (actually pre-proposals) was issued. Proposals totaling $5.73M were received from a total of 84 scientists affiliated with 23 Land Grant Universities and the ARS. $1.55M of these funding requests is for new proposals, and 19 of the applicants are new to the Initiative. The six research area committees are finishing up a formal review process as these proceedings go to print. The Executive Committee will meet with the leaders of the Research Committees on the day before the formal start of the '99 FHB Forum. The Executive Committee will then prepare a proposal for a comprehensive research plan for consideration by the overall Steering Committee the following morning. Once the Steering Committee's input has been incorporated, the overall plan will be submitted to the ARS as the Initiative's formal recommendation for the use of the FY2000 funds. Investigators will need to make any necessary adjustments in their budgets and combine proposals on the basis of investigator to streamline the grant process for ARS. ARS then conducts an internal review process that we expect will endorse the great majority of the recommendations made by the Initiative.

Substantial research efforts were already underway in several states before anyone had notions of a national effort. However, the articles and poster abstracts included in this volume represent a veritable explosion of scab research. Promising new sources of host plant resistance genes are reported, along with substantial progress in molecular tagging of previously discovered genes. Conventional plant breeders are eliminating highly susceptible varieties and improving the resistance in new releases. Likewise, investments in biotech solutions have enabled progress in the arena of genetic engineering. Promising bio-control agents are reported here, and several reports confirm the efficacy of Folicur and possibly other fungicides. Good progress in unraveling the mysteries of the pathogen's life is evident. Some highly innovative methods are being employed in that effort, including employment of *Fusarium* that has a fluorescent gene from jellyfish, and radio controlled aircraft to document aerial spore movement. New knowledge is reported here regarding the fate and effect of DON in food products, as is new work on the toxicological properties of that mycotoxin.

The speed and magnitude of the success our industries have had in generating funds and associated research plans is an arguably unprecedented happening in U.S. plant agriculture. However, all we've really done is enabled ourselves to fully engage in the real challenge, which is the elimination of scab as a destructive force in the U.S. food system. We now have a substantial solution-discovery engine up and running. There are two fundamental challenges deserving of heightened focus during the 1999 FHB Forum and throughout the ensuing year. First, how do we increase the scientific productivity of the resources committed to scab research, and second, how do we minimize the time lag between discovery of a solution and its comprehensive employment in relevant at-risk systems? We believe that we've only begun to scratch the surface of the wealth of opportunities for synergistic collaboration on both of these issues. One means of addressing the first challenge (scientific productivity) is full employment of modern communication technologies, i.e., the Internet. The Internet has already played a significant role in the Initiative's history, both through email list servers, and through a central web-site. However, little if any progress has been made in moving towards the kinds of 'virtual communities' of scientists made possible by the real-time, or near real-time communication systems available through the web. The second challenge, the lab vs. real-world time lag, requires pro-active forward thinking by across-discipline alliances representing all aspects of the continuum from

the lab to the field or factory. In both cases, these challenges require continued investment and belief in research system components that transcend the individual research scientist.

In closing, we wish to commend the ongoing spirit of collaboration that enabled and sustains the U.S. Wheat and Barley Scab Initiative. We are honored to have the opportunity to work in the context of the Initiative and look forward to a productive 1999 National FHB Forum.

Tom Anderson and Rick Ward
Co-Chairs,U.S. Wheat & Barley Scab Initiative

SOME OF MY QUESTIONS, OPINIONS AND EXPECTATIONS ON BREEDING WHEAT FOR SCAB RESISTANCE

Dajun Liu

I feel greatly honored to be invited to attend this forum, which provides me with a rare chance to meet the many scientists involved in the US Wheat & Barley Scab Initiative. Dr. Rick Ward wanted me to speak at this forum, but I have been hesitating what to speak about since it seems that the Chinese scab studies have been frequently introduced and repeatedly documented long ago in this country. But, being a scientist engaging in wheat scab studies in China for more than 20 years, I hope to share with you some of my questions, opinions and expectations on breeding wheat for scab resistance. It would be my great pleasure, if my talk might be of help in your discussions.

I started to touch upon the wheat scab problem in the mid 1970s, when a nation-wide collaborative network of wheat breeding was just under reorganization, and the international exchange of Chinese wheat breeding was just recommenced. At that time, I was involved in the wheat breeding programs south of theYangtze River. In addition to yield and earliness, quite a strong emphasis was placed on scab resistance. Screening nurseries with artificially induced epidemics had been commonly used to determine the response of wheat breeding lines to scab disease. I will never forget my meeting with visitors from CIMMYT in late 1970s at Jiangsu Academy of Agricultural Sciences. Headed by Norman Borlaug, Haldore Hanson, the late Director General of CIMMYT and Glen Anderson, the late Director for Wheat Program of CIMMYT at the time were also in the group. Our guests were shocked by the severity of the scab epidemics in the nurseries. I suppose that the visit might have given them a deep impression about why CIMMYT wheat varieties could hardly be adapted to that part of China. In 1980, I was invited by Norman Borlaug to visit El Batan and Toluca, where I met Dr. Sajaya Rajaram for the first time. We had a vivid discussion about the necessity to also include breeding for scab resistance in the international wheat improvement program at CIMMYT. I was not surprised that CIMMYT initiated a program on scab resistance 2 years later. The cooperation between China and CIMMYT in breeding wheat for scab resistance has significantly strengthened.

Scab resistance always has been an important target of wheat breeding south of Yangtze River. This area consists of more than 10 provinces that grow wheat in China. However, we learned many lessons from the unsuccessful release of improved varieties developed in the early stages of Chinese wheat breeding that relies mainly on selections from landraces and exotic introductions. The breeders of the elder generation in China are well aware of stories related with such lessons. As a result of massive breeding efforts made since 1950s, a number of Chinese cultivars with scab tolerance or resistance were developed and popularized along the middle and lower reaches of Yangtze River. The earliest cultivars were Wan-Nian #2, selected from the improved variety ND 2419 (a cultivar selected from Strampelli's variety Mentana by late Professor Shanbao Jing, the late Directors of the Chinese Academy of Agricultural Sciences and Nanjing Agricultural College, the predecessor of Nanjing Agricultural University) and Wu-Mai #1, a selection from an introduced Italian variety Funo (Stampelli's too). Later, Er-Mai #6, an induced mutatant of ND2419, Su-Mai #2 & #3 and

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Xiang-Mai #1, produced from recombinations between Chinese and Italian genotypes, came out successively from the mid 1960s to mid 1970s. Interestingly, all the above-mentioned varieties with scab resistance or tolerance were developed without exception on the basis of Italian breeding products (such as Metana, Ardito, Giuliari and Funo etc), but a few Chinese landraces were involved. Of greater interest, the Brazilian variety Frontana, generally thought to have Type III resistance to scab disease also involves Italian variety Mentana in its pedigree. It is my belief that there should be some substantial reason explaining such a repeated use of Italian varieties in the international practice of breeding wheat for scab resistance. And it is also true that most of scab resistant or tolerant wheat cultivars or strains released after the 1970s in China (actually also in other countries) almost exclusively derived from the crosses involving Su-Mai #3 and its sister lines or derivatives. My question arose again: " Does it occur reasonably and inevitably?"

There is no doubt of the urgency to broaden the genetic resources for scab resistance used in wheat breeding not only because of restricted genetic basis, but also the inadequate level of resistance. In my view, the extensive and full use of genetic resources from landraces, related species and every possible origin should be strengthened. Of course, to reach the final goal through these approaches may take us a long time. But, a quotation from "Confucianism" might be of instructive importance: " No reach with haste. " It is my opinion that wheat landraces with precisely identified scab resistance from those areas where selection pressure has been existing for centuries, such as southern China and southern Japan, must be adequately evaluated , properly maintained and reasonably utilized. As for the genetic resources for scab resistance from related species and /or of even more remote origins, it seems that great potential will always accompany with increasingly appearing challenges. But, I do think the germplasms with incorporated alien genes shall be able to play unique and irreplaceable roles in breeding wheat for scab resistance in the near future.

In Addition to the germplasms related to breeding scab resistant wheat, I would still like to stress some other restraints that hinder a breakthrough in breeding advancement. To me, how to determine the breeding targets in terms of scab resistance appropriately, how to understand numerous basic aspects of the pathogenesis of the disease and the nature of host resistance better, how to refine the relevant methodology further still need to be studied in greater depth. To pave the way for a breeding breakthrough, continuous achievements in both practical and basic studies are indispensable. Being scientists from developing countries, our expectations of US scientists probably focus more on the basic research.

The reemergence of scab disease of wheat and barley in North America in the early 1990s is, indeed, a disaster for grain production as a whole. But it also brought something beneficial in the renaissance of scab studies on world wide scale. Thanks to the McKnight Foundation, my colleagues and I not only obtained more resources to strengthen our research programs, but also received valuable chance to learn from the recent achievements of the scab studies made in the US and Canada. We are very happy to see the establishment of the USWBSI and its active role. We greatly appreciate your efficient utilization of all the resources you have strived for in recent years. The achievements you made in the development and release of new varieties with improved scab resistance, genetic analysis and gene mapping, pathogen biology, epidemiology, mycotoxin studies have impressed us deeply. Indeed, all these achievements should be a great contribution to the international studies on scab. Concluding my talk I sincerely hope an international initiative to study scab in wheat and barley will soon emerge.

ISOLATION OF TRICHOTHECEN RESISTANT GENES FROM THE WHEAT CULTIVAR FRONTANA

N.J. Alexander[*1], S.L. Ziegenhorn[1,2], G.J. Muehlbauer[3], S.P. McCormick[1], and B. Kurtz[2]

ABSTRACT

Wheat head blight is a plant disease that is caused by the fungus *Fusarium graminearum*. This fungus produces a trichothecene toxin called deoxynivalenol (DON) that acts as a protein synthesis inhibitor and is thought to be correlated with the severity of the disease. It is reasoned that if plants could detoxify DON, then they may exhibit increased resistance to fungal invasion. The wheat cultivar Frontana exhibits limited resistance to infection by *Fusarium graminearum* and it is possible that this resistance is due to the expression of toxin resistant genes. Several cDNA libraries were made from both infected and non-infected wheat heads at differing time periods post-infection, a well as tissue cultures grown with or without DON for varying time periods. For screening, these cDNA libraries were put into a yeast vector and transformed into a yeast strain that is sensitive to the trichothecene toxins. Selection for resistant colonies was then done on toxin media. The resulting inserts were sequenced and compared by BLAST for homology with other sequences in GenBank. Only the fungal gene *TRI101* was isolated from the cDNA libraries made from *Fusarium*-infected wheat heads. This gene codes for an acetyltransferase that has been shown to detoxify a number of trichothecene toxins, including DON. Analyses are continuing on the remaining libraries.

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UPDATE ON DNA MARKERS FOR FUSARIUM HEAD BLIGHT RESISTANCE QTL IN TWO WHEAT POPULATIONS

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OBJECTIVES

Our objectives are to saturate the chromosome 3BS region, previously found to contain a *Fusarium* head blight resistance QTL, with additional DNA markers and develop markers for this region that may be used in a marker assisted selection scheme.

INTRODUCTION

We have identified quantitative trait loci (QTL) for *Fusarium* head blight (FHB) resistance in two wheat populations (Anderson et al., 1998a,b; Waldron et al., 1999). The most significant QTL for FHB was located on the short arm of chromosome 3B and designated *QFhs.ndsu-3B*. The best marker in this region, an AFLP from the primer pair *Eco*RI-agc/*Mse*I-cta explained 17.6% and 15.6% of the phenotypic variation in greenhouse-based evaluation of Type II (Mesterhazy, 1995) FHB resistance in Sumai 3/Stoa and ND2603/Butte 86 recombinant inbred populations, respectively.

MATERIALS AND METHODS

The populations used and methods of evaluation were described previously (Anderson et al., 1998b). Briefly, the populations are recombinant inbred lines from the cross of Sumai 3/Stoa (112 lines) and ND2603 (Sumai 3/Wheaton)/Butte 86 (139 lines). Each population was evaluated twice in the greenhouse for Type II (spread) resistance at North Dakota State University under the supervision of Dr. Robert Stack. The average of the two screenings were used in the analyses reported here.

Primers for the microsatellites (SSR) published by Röder et al. (1998) were synthesized and are being screened for polymorphism among the four parents of these populations as well as parents of other populations. Markers known to be located in putative QTL regions based on our previous research were given the highest priority. PCR amplification was as described by Röder et al. (1998) except 35 cycles of amplification were used instead of 45. Visualization of fragments was by electrophoresis in 5% polyacrilimide gels and silver staining according to the protocol of Bassam et al. (1991).

RESULTS AND DISCUSSION

Of the 142 microsatellite markers screened to date, 48 (34%) were polymorphic between Sumai 3 and Stoa and 79 (53%) between ND2603/Butte 86. The marker *Xgwm264* was mapped using all RIL from the Sumai 3/Stoa population. Unfortunately, this marker putatively mapped to 5BL instead of the expected location on 3BS (data not shown). The marker *Xgwm533* was mapped in the ND2603/Butte 86 population and explained 24.6% of the phenotypic variation in scab resistance in this population (Fig. 1). All lines have been screened with this marker two or three times. Due to discrepancies (2 cases) and apparent presence of heterogeneity in some lines (10 cases), 12 lines are considered missing data for this marker. This marker explains the highest proportion of FHB resistance of those that we have identified to date for a gene inherited from Sumai 3. Unfortunately, this microsatellite was not polymorphic between Sumai 3 and Stoa. Comparison of the interval maps for scab resis-

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tance in these two populations indicates that the scab QTL in these two populations is toward the proximal end of this chromosome with respect to the AFLP locus *XEagcMcta.1* that was mapped in both populations.

Other ongoing research in our lab is directed at 1) obtaining STS markers for the *XEagcMcta.1* AFLP locus; 2) verifying all markers in additional populations; and 3) implementing marker assisted selection in our wheat breeding program. We have cloned the critical fragment from the *XEagcMcta.1* AFLP locus, developed primers, and screened the parents, but have yet to reveal polymorphism.

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Figure 1. Frequency distribution of FHB severity for the ND2603 and Butte 86 alleles at the *Xgwm533* microsatellite locus among 127 ND2603/Butte 86 recombinant inbred lines.

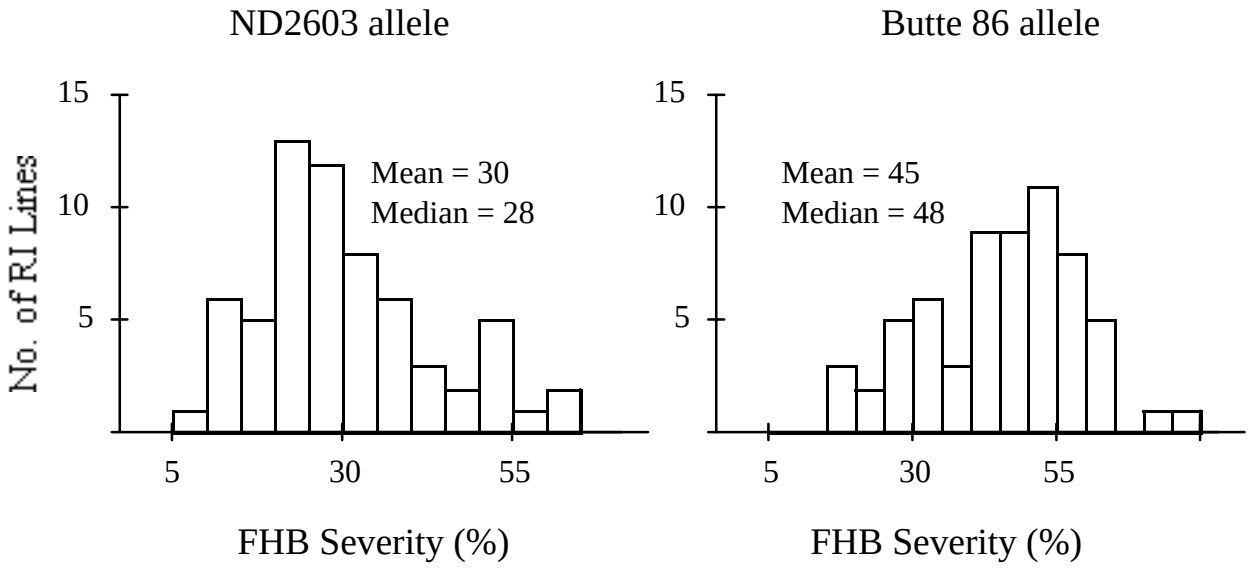
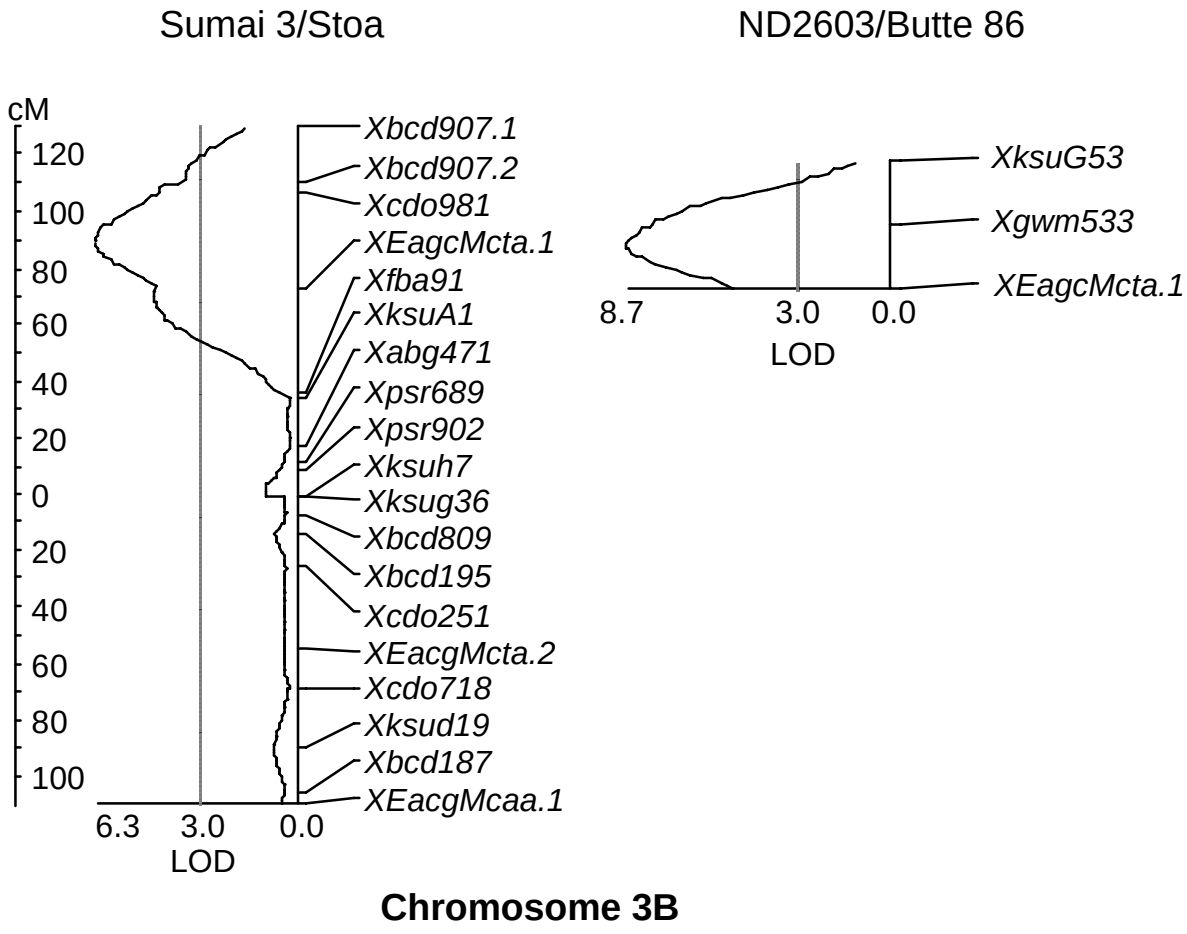


Figure 2. Interval analysis of chromosome 3B *Fusarium* head blight resistance in Sumai 3/Stoa and ND2603/Butte 86 recombinant inbred populations. Chromosomes are aligned at their common marker, *XEag.Mcta.1*. Zero on the cM scale indicates the position of the centromere.



USING AN AFLP MAP TO IDENTIFY SCAB RESISTANCE QTL IN WHEAT

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ABSTRACT

Wheat scab, caused by *Fusarium graminearum*, is a destructive disease in the US. To identify quantitative trait loci (QTL) for scab resistance, we constructed a molecular map using amplified fragment length polymorphism (AFLP) markers with 133 F_9 recombinant inbred lines (RILs). The RILs were derived by single-seed descent from a cross between the resistant wheat cultivar Ning 7840 and the susceptible cultivar Clark. The F_7 to F_{10} RILs were evaluated in the greenhouse for resistance to scab by injecting conidia of *Fusarium graminearum* into a central floret. A genetic linkage map consisting of 27 linkage groups was constructed with 600 AFLP markers. Three QTL, one with a major effect, for scab resistance were identified by interval mapping and collectively explained up to 63% of the variation (R^2). Some of the AFLP markers will be converted into STS markers for marker-assisted selection in wheat breeding programs.

INTRODUCTION

Fusarium graminearum causes scab, an important wheat disease. Scab reduces wheat yield and grain quality. The mycotoxins produced by the fungus in infected grain are detrimental to humans and livestock. The use of resistant cultivars is one way to control the disease (Bai and Shaner, 1994). Scab resistance is a quantitative trait. Phenotypic evaluation of resistance is complicated, laborious, and confounded by environmental variation.

Use of molecular maps to identify quantitative trait loci (QTL) has been reported for important traits in many crops. Once a QTL is identified, molecular markers closely linked to it can be used as tags to selectively transfer the desired genes into different genetic backgrounds by marker-assisted selection. A restriction fragment length polymorphism (RFLP) map has been used to identify scab resistance QTL in 4 wheat chromosomes either from cultivar Sumai 3 or Stoa (Waldron et al., 1999). Recently, we used amplified fragment length polymorphism markers coupled with bulk segregant analysis to identify a major QTL for scab resistance that explained up to 53% of the phenotypic variation (Bai et al., 1999). Our objective in this study was to use an AFLP map to scan the wheat genome for additional QTL controlling scab resistance.

MATERIALS AND METHODS

Recombinant inbred lines (RILs) were derived by single-seed descent from a cross made between the cultivars Ning 7840 (resistant) and Clark (susceptible) in 1990 at Purdue University, Indiana. $F_5 - F_8$ RILs were evaluated for scab resistance in the greenhouse at Purdue University from 1994 to 1996 and the F_{10} RIL was evaluated at the University of Illinois in 1998. Nine to sixteen plants per family were evaluated by injecting 1000 conidia (a mixture of *Fusarium graminearum* isolates from Purdue University) into a central floret of a spike at early anthesis with a hypodermic syringe. To promote infection after inoculation, the plants were placed in a moist chamber for 3 days at 23°-25°C and 100% relative humidity. Spikelets showing symptoms were counted at 3, 9, 15 and 21 days after inoculation. Based on total spikelets per spike,

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counts of blighted spikelets at each of these days were converted to percentage of scabbed spikelets (severity). Final disease severity and area under the disease progress curves (AUDPC), calculated according to Shaner and Finney (1974), were used to characterize the reaction of each plant to infection. Plants from F_{10} families were individually harvested, and seeds were scored for percentage of scabbed kernels (PSK) and weighed to estimate hundred kernel weight (HKW).

DNA was isolated from Ning 7840, Clark, and the 133 F_9 RILs grown in a greenhouse at the University of Illinois using the CTAB procedure (Saghai-Maroof et al., 1984). For each sample, 500 ng genomic DNA was digested simultaneously with *Eco*RI and *Mse*I restriction enzymes. The genomic DNA fragments were ligated to *Eco*RI and *Mse*I adaptors to generate template DNAs for amplification. The *Eco*RI selective primers were labeled with ^{33}P-gATP. PCR was performed in two consecutive reactions in a thermocycler (MJ Research, Inc.). Primers for pre-amplification and selective amplification of genomic DNAs were designed according to Thomas, et al. (1995). Primer combinations showing high levels of AFLP polymorphism between two parents were used to evaluate all 133 RILs. Linkage analysis was performed with Mapmaker (V.2.0 for MacIntosh, Lander et al., 1987) with the Kosambi mapping function and using a maximum recombination fraction of 0.4 and a minimum LOD of 3. For QTL analysis, interval analysis was performed with Mapmaker/QTL (Lander et al., 1987).

RESULTS AND DISCUSSION

Of the 266 AFLP primer combinations tested, about 230 amplified clearly separated band patterns from parental DNA. Each primer combination amplified 70-140 bands, of which 1 to 18 bands were polymorphic. Sixty-three primers with relatively high levels of polymorphism between parents were selected to evaluate 133 RILs. In total, about 600 markers were mapped in 27 linkage groups covering a distance of 2251 cM. The number of markers within each linkage group ranged from 3 to 155. Clustering of AFLP was observed in several linkage groups, including linkage groups 1, 2, 3, 7 and 11. One major QTL for Type II resistance, as reported previously (Bai et al., 1999), was detected in all generations. R^2 for the QTL varied from 30 to 58%. Two other QTL were also detected and present in only some of the generations evaluated (Table 1). These two QTLs had minor effects on percentage of scabbed spikelets and marginally significant LOD values. They were more sensitive to environmental variation than was the QTL in LG12 since they were detected only in some generations.

F_{10} RILs were also evaluated for AUDPC, percentage of scabbed kernels and hundred kernel weight. The major QTL in linkage group 12 affects not only visual symptoms on the wheat spikes, but also seed infection. In the F_{10} the major QTL explained 53% to 58% of variation for the four scab measurements (Table 2). In breeding programs, this major QTL is an ideal candidate for marker-assisted selection. Four of the markers closely linked to the QTL were cloned and will be sequenced to develop STS markers. Interval mapping detected an additional QTL in linkage group 21 in the F_{10} RIL for AUDPC (Table. 2). This QTL was also associated with percentage of scabbed seeds and hundred seed weight, and therefore appears to have an effect on more than visual symptoms on spikes.

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Table 1. R^2 and LOD values of QTL for percentage of scabbed spikelets as detected by interval mapping for 5 RIL generations in the greenhouse

LG[a]	F_5		F_6		F_7		F_8		F_{10}	
	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD
LG12	33.9	11.1	30.4	10.2	55.9	23.4	30.3	9.9	57.7	22.4
LG13	8.6	2.4	8.8	2.1	8.4	2.5	-	-	-	-
LG17	-	-	-	-	7.3	2.1	-	-	8.1	2.4
Total[b]	42.9	14.7	38.8	12.5	61.9	26.3	30.3	9.9	60.7	23.2

a. Linkage group number
b. Total variation explained by all the significant QTL detected by interval mapping and its LOD value.

Table 2. R^2 and LOD values of QTL for percentage of scabbed spikelets (PSS) at 21 days after inoculation, area under disease progress curve (AUDPC), percent scabbed kernels (PSK), and hundred kernel weight (HKW) as detected by interval mapping for 5 RIL generations in the greenhouse

LG[a]	PSS		AUDPC		PSK		HKW	
	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD
LG12	57.7	22.4	55.4	21.5	57.7	21.7	52.8	19.0
LG17	8.1	2.4	7.5	2.2	-	-	-	-
LG21	-	-	12.3	2.4	12.5	2.4	17.0	3.0
Total[b]	60.7	23.2	58.1	22.2	63.2	23.1	59.2	22.0

a. Linkage group number
b. Total variation explained by all the significant QTL detected by interval mapping and its LOD value.

IDENTIFICATION OF GENES FOR RESISTANCE TO PATHOGENS IN NON-HOST PLANTS

Albert H. Ellingboe

ABSTRACT

Control of plant diseases via breeding disease resistant cultivars has been considered to be environmentally safe and cost effective. Control of disease by breeding is usually a never-ending endeavor because pathogens are living organisms capable of genetic changes that render the genes for resistance ineffective. A common observation is that there are not sufficient genes for resistance in the economic species so it has been necessary to introgress genes from related species into economic species. The proposal submitted to the USDA is to use a strategy devised for the specific purpose of identifying specific genes for resistance to a pathogen, *Fusarium moniliforme*, in a non-host species. The procedure uses a pathogen of the plant species to be mined as a vehicle to study the individual genes in the pathogen for which genes for resistance are sought. Once the specific genes in pathogen and host that control the interactions are identified, the procedures to clone the genes, and transfer the resistance genes to wheat and barley, will follow established procedures.

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AFLP LINKAGE MAP OF *GIBBERELLA ZEAE*

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ABSTRACT

A genetic map of *Gibberella zeae* could be useful in population genetics studies, map-based cloning of interesting genes, QTL analysis, and comparisons with related species. Complementary *nit* mutants of *G. zeae* strains R-5470 (from Japan) and Z-3639 (from Kansas) were crossed according to previously described methods. 99 *nit*+ progeny were selected and analyzed for polymorphisms using AFLP analysis. Analysis of thirty-three two-base selective primer pairs were analyzed revealing 1084 polymorphic loci of which 1039 unambiguously segregate into 9 linkage groups. The total map length of the genome from this analysis is estimated to be in excess of 2800 cM with an average interval of 2.7 map units per locus. Three linkage groups exhibit a high degree of segregation distortion. Selection of *nit*+ progeny may account for some but not all of the segregation distortion in the cross.

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ENHANCED SCAB RESISTANCE IN WINTER WHEAT GERMPLASM BY PLANT TRANSFORMATION

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ABSTRACT

Fusarium graminearum is an important and emerging pathogen of wheat not controlled effectively chemically or by genetic resistance in the world. Losses have occurred to the extent that wheat production in some parts of the US and Canada is severely threatened. Definition of the genetic and biochemical bases of the fungal pathogenesis may lead to the development of control strategies, but may take a significant period of time. Expression of known and novel antifungal genes in transgenic wheat, on the other hand, may provide quick, effective and durable scab control strategies. The ability to create transgenic wheat resistant to this fungus has significant impact to growers. We have identified two different types of genes, antifungal lactoferrin and antiapoptic genes, for resistance to the scab fungus. An in-vitro test indicated effectiveness of lactoferrin against the scab fungus. Similarly, transgenic tobacco plants expressing the antiapoptic genes were immune to a number of necrotrophic fungi. *Fusarium graminearum* is a necrotrophic fungus. In order to improve wheat transformation efficiency, a comparative study was conducted to ascertain co-expression, integration patterns and inheritance ratios of transgenes in wheat transformants derived from microprojectile bombardment and *Agrobacterium*-mediated transformation protocols. A total of 9 and 7 independent events were derived from the *Agrobacterium*-mediated and microprojectile systems, respectively. Wheat transformants were analyzed at either the R1 or R2 generation. Co-expression of the non-selected transgene (GUS) was observed in leaf tissue in 5 out of the 9 Agrobacterium-mediated events and 1 out the 7 microprojectile events. Co-expression in floral tissue was observed in 6 out of the 9 *Agrobacterium*-mediated events and 5 out of 7 microprojectile events. Although co-expression was observed at a higher frequency in the *Agrobacterium*-mediated transformants the 1 microprojectile event which GUS co-expression was observed in leaf, the expression level was significant higher than the 5 Agrobacterium-mediated events. Southern blot analysis on progeny revealed all 9 Agrobacterium-mediated events possessed 1 to 2 loci with 1 to 2 copies per locus, while 2 microprojectile events had fragmented copies with 1 extremely complex integration pattern.

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WHEAT SPIKE-*FUSARIUM GRAMINEARUM* MOLECULAR INTERACTIONS

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ABSTRACT

Fusarium Head Blight (FHB) of wheat is a crippling disease that causes severe economic losses in many of the wheat growing regions of the world. Our group is studying the interactions between wheat spikes and *F. graminearum* during infection. Microscopy of inoculated glumes revealed that the fungus appeared to penetrate through stomata, exhibited subcuticular growth along stomatal rows, colonized glume parenchyma cells and sporulated within 48-76 hours after infection (hai). No major differences in the timing of these events were found between Sumai 3 (resistant) and Wheaton (susceptible) genotypes. To study the host response to infection, we examined the expression of six defense response genes (peroxidase, PR-1, PR-2, PR-3, PR-4, and PR-5) in resistant (Sumai 3) and susceptible (Wheaton) genotypes during the initial 48 hai. In both genotypes, transcripts of the six defense response genes accumulated as early as 6-12 hai and peaked at 36-48 hai. Greater and earlier PR-4 and PR-5 transcript accumulation was observed in Sumai 3 as compared to Wheaton. These data indicated that wheat responds to infection by inducing a set of defense response genes. In a companion study, we examined whether infection induced a systemic response of the defense response genes. We found that infected plants of both resistant (Sumai 3) and susceptible (Wheaton) genotypes induced defense response genes in uninfected portions of the wheat spike, indicating that wheat mounts a systemic reponse to infection. Our results provide the first documentation of the infection pathways on wheat glumes and provide the first look at the host response at the molecular level.

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ENHANCED RESISTANCE TO SCAB BY GENETIC ENGINEERING WITH GENES FOR DEFENSE PROTEINS

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ABSTRACT

Pathogenesis-related proteins represent inducible defenses that help plants fight pathogen and pest infestations. This inducible defenses include biochemically diverse proteins fungal cell wall hydrolyzing enzymes (e.g. chitinases and beta -1,3-glucanases), inhibitors and membrane-permeabilizing proteins (e.g. thaumatin-like proteins, TLP). Genes/cDNA's for several of these proteins have been isolated from Rhizoctonia-infected rice and scab infected wheat in our laboratories. We have introduced some of these cDNA's/genes into calli derived from immature wheat embryos by biolistic procedures and regenerated transgenic plants from them. The regenerated plants were shown to possess the gene by Southern blotting and to express the transgenes by enzyme assays and western blotting using appropriate antisera for chitinase, -1,3-glucanases and TLP. One line of transgenic wheat plants expressing high levels of TLP at high level were tested for resistance to scab by the single floret inoculation method and were found to have significantly greater resistance to scab compared to control plants. Work is currently in progress to introduce combinations of chitinase, beta -1,3-glucanases and TLP to identify specific combinations of these pathogenesis-related proteins that would have the highest level of resistance to scab.

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EXPRESSION OF CANDIDATE ANTI-*FUSARIUM* PROTEIN GENES IN HEXAPLOID WHEAT

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OBJECTIVES

The aims of our project are to generate transgenic lines of hexaploid wheat (*T. aestivum*) expressing genes that encode candidate antifungal proteins, and to optimize the activities of the antifungal proteins in wheat for effectiveness against scab.

INTRODUCTION

Efforts are ongoing in laboratories throughout the world to obtain transgene-mediated resistance to *Fusarium* head blight in wheat, barley, and oat. Recently, Gill and coworkers reported that the expression of a rice thaumatin-like protein delayed the development of scab in transgenic wheat lines (Chen *et al*. 1999).

We transformed wheat with six genes encoding candidate anti-*Fusarium* proteins with different modes of antifungal action. The proteins are *Fusarium* DON acetyltransferase (from *FsTRI101*, McCormick *et al*., in press), *S. cerevisiae* multidrug transporter (from *PDR5*, Balzi *et al*. 1994), wheat thaumatin-like protein (from *tlp-1*, Rebmann *et al*. 1991), *Fusarium* glucanase (FvGlu), *Fusarium* endochitinase (FvChi1), and *Fusarium* exochitinase (FvChi2). The coding sequences of the genes are under constitutive regulation by the maize *Ubiquitin-1* promoter (Christensen and Quail 1996). A *Bgl*II restriction site in the original *Ubi-1* promoter was removed to facilitate the cloning of the coding sequences at the remaining *Bgl*II site in our monocot expression vector, pUBKBglII-. In this poster, we 1) present the pattern of GUS activity conferred by the modified *Ubi-1* promoter in wheat floret organs, 2) summarize the results of our transformation experiments, 3) discuss progress in quantifying transgene copy numbers and expression levels in wheat endosperm, and 4) discuss codon usage in candidate genes from fungal sources.

MATERIALS AND METHODS

Removal of the *Bgl*II restriction site in the *Ubi-1* promoter region of pUBK was carried out using the QuikChange™ Site-Directed Mutagenesis Kit and protocols (Stratagene, La Jolla, CA). Wheat transformation of cv. Bobwhite was carried out as described in Weeks *et al*. (1993), with modifications (Blechl and Anderson 1996). Floret organs of transformants expressing *uid*A under regulation of the modified *Ubi-1* promoter (lines B70-14b, AB7-203b, AB7-148) were assayed for GUS activity by histochemical staining (Gallagher 1992, Weeks *et al*. 1993). Leaf genomic DNA was obtained from a preparation of enriched nuclei (D'Ovidio *et al*. 1992). Total DNA was prepared from small leaf sections (Dellaporta *et al*. 1983). Total RNA was isolated from endosperm of homozygous transgenic lines at 15 to 25 days post-anthesis (Altenbach 1998). DNA and RNA blot analyses were done as described in Sambrook *et al*. (1989), using Zeta Probe nylon membrane (Bio-Rad, Hercules, CA). Hybridization probes consisted of coding sequence fragments that were radiolabeled with ^{32}P-a-dCTP. Codon usage data was generated using the Codon Usage Database at Kazusa (http://www.dna.affrc.go.jp/~nakamura/codon.html).

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RESULTS AND DISCUSSION

The modified *Ubi-1* promoter conferred high transient levels of GUS activity in triticale endosperm bombarded with *Ubi*::*uid*A (A. Blechl and G. Sharma, unpub.), and its activity was indistinguishable from that of the unmodified *Ubi-1* promoter in pACH15 (Christensen and Quail 1996). To characterize the modified *Ubi-1* promoter in wheat florets at anthesis, we examined GUS activity in several *Ubi*::*uid*A transformants. GUS activity was found primarily at the base and tips of the glume, lemma, and palea, and in pollen, brush and endosperm tissue, but not in the anther. Therefore, the modified *Ubi-1* promoter can confer gene expression in organs that are potential barriers to the spread of scab in the head.

Using transformation methods developed in the laboratory, we generated 31 transgenic lines of wheat carrying one of six candidate genes (Table I). To rapidly and accurately identify lines carrying the candidate transgenes, we used PCR to amplify transgene DNA from small amounts of leaf tissue. This approach was useful for identifying homozygotes and for following transgene inheritance in lines in which the bialaphos resistance marker gene was inactivated. To date, there are 2 to 11 lines representing each candidate gene, and a total of 16 lines homozygous for the candidate gene.

Table I. List of candidate genes and transgenic wheat lines.

Gene Name	Lines	Homozygotes	mRNA Detected
FsTRI101	4	3	in 1 of 3 tested
PDR5	11	6	in 0 of 4 tested
tlp-1	3	1	in 1 tested
FvChi1	8	4	in 1 of 3 tested
FvChi2	2	1	in 0 of 1 tested
FvGlu	3	1	in 1 tested

Gene Name	Mode of Action	Reference
FsTRI101	DON inactivator	McCormick, in press
PDR5	DON exporter	Balzi, 1994
tlp-1	Membrane permeabilizer	Rebmann, 1991
FvChi1	Chitin degradation	unpub.
FvChi2	Chitin degradation	unpub.
FvGlu	Glucan degradation	unpub.

Genomic DNA blots of four *FsTRI101* lines and three FvChi1 lines showed one to four integrated, intact copies of the candidate genes. In all but one of these lines, additional bands were present, indicating rearrangements. Expression of candidate mRNAs in the endosperm (~15-25 dpa) of available homozygous lines was determined using RNA blots. Our most highly-expressing line so far accumulated abundant levels of the wheat *tlp-1* mRNA in the endosperm. The remaining two *tlp-1* lines will be examined when homozygotes are identified. Expression of the fungal genes was generally lower. mRNAs of the expected sizes were detected in endosperm RNA from the homozygote carrying the FvGlu gene, from one of the four *FsTRI101* homozygotes, and from one of three FvChi1 homozygotes (Table I). None of the four *PDR5* lines tested to date accumulated detectable amounts of mRNA in the endosperm. Progress with RT-PCR, a more sensitive method for RNA detection, will be reported in the poster.

Genes from microbial and plant sources often differ in nucleotide composition, codon usage and other features that affect the expression of genes in a foreign host. Our initial computerized analyses indicated differences in codon usage of the candidate *Fusarium* genes as compared to a compilation of wheat genes (Table II). We speculate that the low levels of specific mRNAs in lines containing the fungal genes might be due to such differences. Codon usage analyses are under way for cereal genes expressed in endosperm, pollen and leaf, and for *Fusarium* genes. If necessary, strategies will be devised to modify the fungal genes for higher mRNA stability and translation (e.g., Iannacone *et al*. 1997; Lonsdale *et al*. 1998) in wheat floral tissues.

Table II. Third nucleotide frequencies in codons of *F. venenatum* candidate genes and wheat (*T. aestivum*) genes

A. FvGlu [a/] Amino acid	C,G	A,U	CG/AU Ratio [b/]	*T. aestivum* Ratio [c/]
F	15	0	>15	1.88
K	29	0	>29	4.36
N	14	1	14.0	2.37
Q	13	1	13.0	0.88
B. FvChi1 [d/] Amino acid	C,G	A,U	CG/AU Ratio [b/]	*T. aestivum* Ratio [c/]
G	11	28	0.4	1.70
K	14	1	14.0	4.36
N	20	4	5.0	2.37
R	4	11	0.4	2.33
C. FvChi2 [e/] Amino acid	C,G	A,U	CG/AU Ratio [b/]	*T. aestivum* Ratio [c/]
K	2	3	9.7	4.36

[a/] Contains a total of 302 codons; [b/] Ratio of codons NNC/G to NNA/U encoding the specified amino acid; [c/] Total of 119,403 codons from 353 wheat genes (www.kazusa.or.jp/codon/); [d/] Contains 400 codons; [e/] Contains 582 codons

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MOLECULAR MAPPING FOR *FUSARIUM* HEAD BLIGHT IN A RICL POPULATION OF TETRAPLOID WHEAT

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ABSTRACT

The extensive damage caused by *Fusarium* head blight (FHB) has made it necessary to develop resistant lines of durum and common wheat. Unlike hexaploid wheat, there are no useable sources of resistance in domesticated tetraploid cultivars. One species that shows promise as a source for FHB resistance is an accession of *Triticum turgidum* L. var. *dicoccoides* (Joppa, 1997). Langdon-dicoccoides disomic substitution lines [LDN(Dic)] were analyzed for Type II resistance. The Langdon durum line with a pair of chromosomes from an accession of *Triticum turgidum* L. var. *dicoccoides* [LDN(Dic-3A)] was shown to have reduced infection to FHB relative to the parents (Elias et al., 1996). A Recombinant Inbred Chromosome Line (RICL) population of 83 individuals derived from LDN(Dic-3A) has been analyzed over three seasons. These data, molecular marker (RFLP, AFLP, and microsatellite) mapping data and QTL analysis results futher delineating the location of FHB resistance loci on 3A are presented.

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TARGETING OF ANTIFUNGAL GENES TO INHIBIT GROWTH OF *FUSARIUM GRAMINEARUM* IN BARLEY

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OBJECTIVES

The objective of this research is to produce *Fusarium*-resistant barley through genetic transformation with specifically targeted native antifungal protein genes. Studies will examine the tissue preference and subcellular route(s) of Fusarium infection. This will guide strategies for future gene expression targeting. Tissue-specific promoters will be developed for lemma/palea and pericarp. Expression vectors will be developed to target antifungal genes (particularly hordothionin) to the intracellular space.

INTRODUCTION

Since 1993, a sustained *F. graminearum* epidemic has destroyed much of the U.S. barley and wheat crops. The production of mycotoxins (e.g., DON) by *Fusarium* makes the harvest unsuitable for food, feed or malting. At present, there are no known barley cultivars with biochemical resistance to *Fusarium*, although some have various levels of tolerance and avoidance. Thus, genetic transformation may be the only method for introducing biochemical resistance. This research seeks to redirect the expression of native genes encoding antifungal seed proteins so that they will be produced in the path of invading Fusarium hyphae.

Research is needed to determine the tissue preference and subcellular route of *F. graminearum* infection. Antifungal proteins must be expressed in the most appropriate tissue and subcellular compartment to avoid placing a metabolic burden on the plant and to minimize pressures which select for resistant pathogen strains. There is very little literature on microscopic studies of penetration by the *Fusarium* genera. In a review of *Fusarium* blight (Parry et al., 1995), the only histological study of *F. graminearum* (Pugh et al., 1933!) noted that hyphae can penetrate wheat kernel tissues both inter- and intracellularly. *F. oxysporum*, which causes root rot in flax, colonizes root cap cells through inter- and intracellular penetration (Kroes et al., 1998). Hyphae grow through the middle lamella and cause collapse of adjoining cells, prior to penetration. Early events in the infection of cotton root tip cells by *F. oxysporum* include the formation of a thin infection peg, without accompanying cell lysis (Rodriguezgalvez and Mendgen, 1995). The penetration of wheat stem cells by *F. culmorum* was shown to involve a stage in which hyphae penetrate the wall perpendicularly and then grow some distance through the intracellular space (Ebrahim-Nesbat et al., 1991). Our research, with a strain of *Fusarium* (gfp/Fusarium) transformed with the green fluorescent protein (gfp) gene of jellyfish, shows that when *Fusarium* colonization occurs on the lemma, hyphae can grow rapidly to the tip of the floret and colonize the extruded anthers or (later) stigmatic and ovary epithelial hairs. Hyphal growth rapidly proliferates downward and covers the pericarp. Hyphae can also penetrate the lemma and grow directly into the pericarp within two days from spore inoculation. It is therefore essential to express antifungal genes in these tissues.

Plants synthesize a variety of pathogenesis-related proteins (PRPs; Linthorst, 1991), often in

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response to pathogen invasion. While the mechanism of PR-1 proteins is unknown, PR-2 proteins include beta-(1,3)-glucanases, PR-3 proteins include chitinases, and PR-4 proteins bind chitin of hyphal cell walls (Hejgaard, 1992). PR-5 proteins are comprised of osmotin (Singh et al., 1987), PRHv-1 (Hahn et al., 1993), PWIR2 (Rebmann et al., 1991) and other thaumatin-like proteins (TLPs). Permatins are TLPs found in many cereals and are homologous with other PR-5 proteins. Permatin has been purified from maize and found to have antifungal properties (Roberts and Selitrennikoff, 1990). An additional group of PRPs is the highly basic low molecular weight thionin proteins.

Barley leaves and roots produce thionins related to seed hordothionin (HTH). These can be induced by fungi and have antifungal properties *in vitro* (Florack and Stickema, 1994). Barley leaf thionins are associated with the vacuole and cell wall (Reimann-Phillip et al., 1989). Both were shown to be toxic to the fungal pathogen of sugar cane *T. paradoxica* at 2.5 mg/ml. The Arabidopsis *Thi2.1* thionin gene is inducible by pathogenic fungi, and its constitutive over-expression leads to resistance to *F. oxysporum* (Epple et al., 1997). HTH has been expressed in tobacco and enhanced resistance to pathogenic fungi in some studies but not in others (Carmona et al, 1993; Epple, et al., 1997). Despite having these properties, the non-seed thionins apparently do not protect barley from *F. graminearum.* Low concentrations (ca. 5 mg/ml) of hordothionin completely suppress germination and growth of *F. graminearum* spores (unpublished data). However, HTH is found only in the starchy endosperm and is not available to prevent colonization by *Fusarium* on exposed floret tissues.

It is necessary to understand the mechanism of HTH targeting so that it can be rerouted to the intracellular space through the secretion pathway. Leaf thionins are synthesized as 17.4 kDa precursor proteins on membrane-bound polysomes (Ponz et al., 1983). The precursor protein contains an N-terminal signal peptide which facilitates transport into the ER lumen. A C-terminal acidic protein (AP) that constitutes about half of the precursor is processed away, leaving a mature protein of only 5 kDa. In leaves of tobacco transformed with barley HTH, the signal peptide of HTH was found to be essential for expression at the protein level, and the AP was required for high-level expression (Florack et al., 1994). HTH was not secreted into the intercellular spaces but was found mainly in the microsomal (vacuolar) and membrane fractions. The AP may not be involved in targeting in the leaf; the targeting signal might lie within the mature protein. Processing and targeting in the seed may be similar to that in the leaf. HTHs may be associated with protein bodies, since these are derived from the ER, as are vacuoles. Ponz et al. (1983) presented evidence that they are not associated with protein bodies but are instead extrinsically associated with the ER. However, earlier research determined that HTH is externally associated with protein bodies and is part of the protein-lipid matrix in which they are embedded (Carbonero et al., 1980).

MATERIALS AND METHODS

Route of Fusarium invasion

A strain of *F. graminearum* transformed with gfp has been produced by Tom Hohn (Novartis, NC (formerly at USDA, Peoria, IL)). The hyphae and spores are readily viewed by their green fluorescence with a Zeiss dissecting microscope equipped with a short wave blue light source. Several methods have been explored in this lab for visualizing the initial penetration events. Standard paraformaldehyde fixing and paraffin embedding techniques techniques failed because they destroyed gfp fluorescence. Cryostat sectioning preserved fluorescence, but spore attachments were apparently disrupted during

sectioning. A simpler technique has been devised whereby lemmas are infected for 6 h and then peeled into fine tissue strips. This preserves a high frequency of conidiospore attachments. These will be viewed by confocal microscopy (Keck Neural Imaging Center, Univ. of Wisconsin). Penetration by the fluorescent hyphae will be analyzed by processing of serial images at multiple depths of focus (Z-series).

Screening for tissue-specific genes and production of promoters

The differential display technique (Liang and Pardee, 1992) is being used to detect genes expressed only in lemma and palea tissues or in the pericarp, using flag leaves as a control. RT-PCR reactions are conducted with 5'-end RAPD primers and ETVN 3'-end primers. ^{35}S-labeled products are separated by PAGE. Bands which appear in the spike lanes and not in leaf lanes are excised, reamplified, labeled with ^{32}P and used to probe RNA (northern) blots of lemma/palea, pericarp and leaf RNAs. Tissue-specific gene candidates will be cloned and sequenced, and the corresponding nuclear genes will be purified from a Morex genomic BAC library. This service is being generously provided by Andy Kleinhofs (Washington State U.). The deduced promoter regions will be subcloned and ligated in front of the gfp gene (provided by Jen Sheen, Harvard) to test promoter activity and tissue-specificity.

Vector construction and expression analysis

Transient expression studies of promoter activity will be conducted with the pAHC17 vector, which contains the Ubi promoter (and first intron), but has no selectable marker. Gfp has been inserted behind the Ubi promoter, so that it is expressed in barley and in stably transformed oat (Kaeppler et al., 1999). Vectors for stable expression of gfp and antifungal genes were prepared from the Ubi/GUS+Ubi/BAR vector pAHC25 by replacement of the GUS gene. Barley promoters will replace the Ubi promoter. The modified vectors will be attached to gold particles and used to transform barley through the biolistic (gene gun) approach (Wan and Lemaux,1994). Screening of putative transformants will be conducted using PCR on leaf DNA extracted by the CTAB procedure. Particle bombardments of isolated lemmas, pericarps and leaves will be conducted with candidate tissue-specific promoters, linked upstream from gfp. Studies will also be conducted to determine the optimal developmental stages for transient promoter/gfp reporter expression in each tissue, and bombardment conditions will be optimized for each to ensure valid tissue comparisons. If a promoter proves to be tissue-specific, promoter/HTH fusions will be constructed. Resulting transformants will be tested for *Fusarium* resistance.

Subcellular targeting of HTH

In order to target HTH to the intracellular space and/or to the secretion pathway, three constructs will be synthesized. These will be inserted into the pAHC25 expression vector in place of the GUS gene. Construct 1 will attempt to divert targeting from the vacuole by inserting a KDEL ER retention signal (Gomord and Faye, 1996) between the N-terminal signal peptide (SP) and GUS. It is predicted that the mature HTH-GUS fusion will be directed to the intracellular space by bulk flow. Construct 2 will contain the SP and mature protein, followed by GUS. Without the AP, the HTH-GUS fusion may be directed to the intracellular space. Construct 3 (control) will contain the SP, followed only by the GUS sequence. Construct 4 will contain the barley high-pI a-amylase signal sequence, preceding the HTH and GUS. This signal has been shown to direct gfp secretion in barley leaves (Nielson et al., 1999). GUS will be used as a reporter gene, instead of gfp, since much of gfp tends to localize at the nucleus. HTH components for these

constructs were subcloned from our full-length a-hordothionin clone, Thio12 (identical to barley genomic Hth-1,GenBank accession M23080), after removing introns. GUS was subcloned from the GUS gene in pAHC25. An oligonucleotide primer primer encoding KDEL has been synthesized. An oligonucleotide encoding the barley high-pI a-amylase signal sequence will be synthesized from the corresponding sequence in cDNA clone pM/C (Khursheed and Rogers, 1988). Constructs will be inserted into pAHC25 behind the Ubi promoter and intron. The vectors will be delivered into lemmas by particle bombardment. Subsequent compartmentalization will be assessed after 48 h by staining for GUS activity. Paraffin-embedded sectioned tissues will be viewed under oil immersion. If HTH-GUS is successfully targeted to the intracellular space, the targeting signal will be incorporated behind the our tissue-specific promoters.

RESULTS AND DISCUSSION

A previously unknown gene, D5, has been cloned and found to be expressed only in the lemma/palea tissues of the spike (Sathish et al.,1999). The promoter was subcloned from a BAC clone, provided by Andy Kleinhoffs. This was shown to drive the expression of a gfp reporter gene in a D5/gfp construct. In transient assays, expression was found in lemmas and not in leaves. We have successfully transformed Golden Promise with our cloned full-length HTH cDNA clone, thio12, and have so far found five transformants that produce HTH mRNA in seedling leaves. We have also produced transformants carrying the gene for another antifungal permatin protein, BARPERM1 (Nuutila et al., 1998; Skadsen et al., 1999). We are currently studying the expression of the *Barperm1* gene in developing seeds of untransformed barley. These studies showed that the permatin encoded by the Barperm1 gene is localized in the aleurone and ventral furrow of developing seeds (Sathish and Skadsen, ms. submitted).

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EXPLORING THE MOLECULAR MECHANISM OF FUSARIUM HEAD BLIGHT RESISTANCE AND DEVELOPING BREEDER-FRIENDLY DNA MARKERS TO FHB FOR WHEAT IMPROVEMENT

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OBJECTIVES

The goal of this project is to develop a molecular understanding of FHB development and to reveal the molecular mechanism of FHB resistance in wheat, while identifying breeder-friendly, PCR-based DNA markers for indirect selection of the FHB resistance in our breeding program. Our specific objectives in the past year were: 1) optimizing protocols for mRNA differential display and for AFLP; 2) assaying some newly introduced Chinese wheat lines for FHB resistance; 3) screening AFLP polymorphism among the parent lines; and 4) constructing mapping populations. Our objectives in the coming year will be: 1) identifying genes whose transcription is related to FHB development and/or to FHB resistance; 2) cloning, sequencing and analyzing the ESTs of the identified genes of interest; 3) continuing development of mapping population; and 4) screening the mapping population for AFLP markers for FHB resistance.

INTRODUCTION

An efficient way to battle FHB is to control it genetically. However, a major limiting factor is the lack of information about FHB resistant genes and resistance mechanism, especially at the molecular level. Adding to the difficulty associated with the utility of linkage-based DNA markers is the fact that FHB resistance in wheat is basically additive and is quantitatively inherited. In this project, we will first try to identify differentially expressed wheat and fusarium genes related to FHB development and/or FHB resistance in wheat, and then to clone, sequence and characterize the genes of interest or their expressed sequence tags (ESTs). Finally, we will investigate the physiological and biochemical pathway(s) that are involved as well as the physical locations where the genes are expressed. We expect to discover at the molecular level how wheat interacts with the scab pathogen and how Type II resistance inhibits FHB development.

Although the National Wheat and Barley Scab Initiative did not fund this project in FY1999, preliminary work has been initiated. We evaluated some newly introduced Chinese spring wheats for FHB resistance in the greenhouse. We have started investigating differentially expressed wheat and *Fusarium* genes between the FHB-susceptible and -resistant parents during FHB development (Figure 1). Searching for AFLP markers to FHB resistance has been initiated.

METHOD AND MATERIALS

Newly introduced Chinese wheat lines Yiyuan 2, Chuanyu 12, 10A, 88-1643 and 87-429 were evaluated for FHB resistance. Sumai 3 and Wheaton were used as controls. FHB evaluation was done in the greenhouse by spike spraying and floral inoculation. FHB severity, kernel yield/spike, and kernel quality were examined. Sumai 3 and Yiyuan 2 were also screened for AFLP polymorphism against Wheaton. Sumai 3, Wheaton, SS5 (a hybrid-derivative from a wheat intermediate wheatgrass cross) and LMPG-6 were used in the study of the differential display.

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High-quality, non-degraded total RNA was isolated from the samples with the guanidine hydrochloride extraction method (Logemann et al. 1987). The SuperScript RT-PCR kit (Life Technologies, Gaithersburg, MD, USA) was used to synthesize the first-stand cDNA. For the mRNA differential display (Liang and Pardee,1992; Welsh et al. 1992), the RNAimagetm kit from GenHunter (Nashville, TN, USA) was used and the protocol provided by the manufacturer was followed with necessary modifications. PCR products were separated on 6% denatured polyacrylamide sequencing gels and visualized by silver staining (Lohmann et al. 1995).

Genomic DNA was isolated with DNAzol ES (Molecular Research Center, Cincinnati, OH). A silver-staining-based AFLP protocol was developed on the basis of AFLP Analysis System (Life Technologies, Grand Island, NY) and DNA Silver Staining System (Promega, Madison, WI), and used to screen polymorphic loci among parental lines (Xing et al. 1999).

RESULTS AND DISCUSSION

Our preliminary FHB assay showed that Yiyuan 2, Chuanyu 12 and 10A showed some degrees of Type II resistance with an average FHB severity of 2.67, 1.38 and 1.86, respectively (Yen and Xing 1999). Our preliminary data also indicated that Yiyuan 2 may also have usable Type III and Type V resistances. Yiyuan 2 was crossed with susceptible line Wheaton and the hybrid populations have been advanced to F2.

Figure 1 shows an example of our mRNA differential display between FHB-resistant and FHB-susceptible wheat lines. We are now optimizing the protocol and will use it to discover differentially expressed ESTs between the FHB-susceptible and the FHB-resistant parents this winter. We plan to identify, clone, sequence and analyze the ESTs of interest. Sequence information obtained will be used to clone the full-length cDNAs and then the genes of both wheat and *F. graminearum* that are related to FHB development or resistance. Analyses of the cloned cDNAs or genes may greatly increase our knowledge on molecular pathogenesis of FHB and molecular mechanism of FHB resistance as well as on the molecular interaction between the pathogen and wheat, hence enhancing our ability to control FHB epidemics. The cloned ESTs will also be converted to PCR-based markers for indirect selection of FHB resistance.

Our preliminary AFLP assay with 51 PCR primer sets revealed a total of 587 and 686 polymorphic loci for the Yiyuan 2-Wheaton and the Sumai 3 - Wheaton pairs with an average of 11.5 and 13.5 polymorphic loci per primer set, respectively. Figure 2 shows an example of the AFLP polymorphism between Yiyuan 2 and Wheaton. We will start to screen the RILs for FHB-linked AFLP loci in the coming year. Identified AFLP loci will then be converted to PCR based markers for indirect selection for FHB resistance in our breeding program.

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Figure 1. (Right) An example of the mRNA differential display. The mRNA samples from FHB-free young seedlings were compared with differential display between FHB-resistant wheat SS5 (left) and FHB-susceptible wheat LMPG-6 (right). The mRNA differential display was done with the RNAimagetm kit from GenHunter (Nashville, TN) with the primer set of H T11C/H-AP2. The PCR products were separated on 6% denatured polyacrylamide sequencing gel and visualized with silver staining.

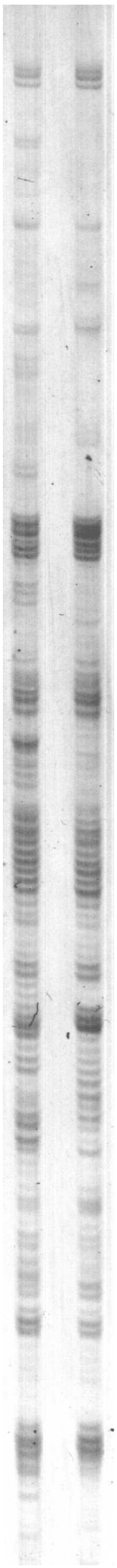
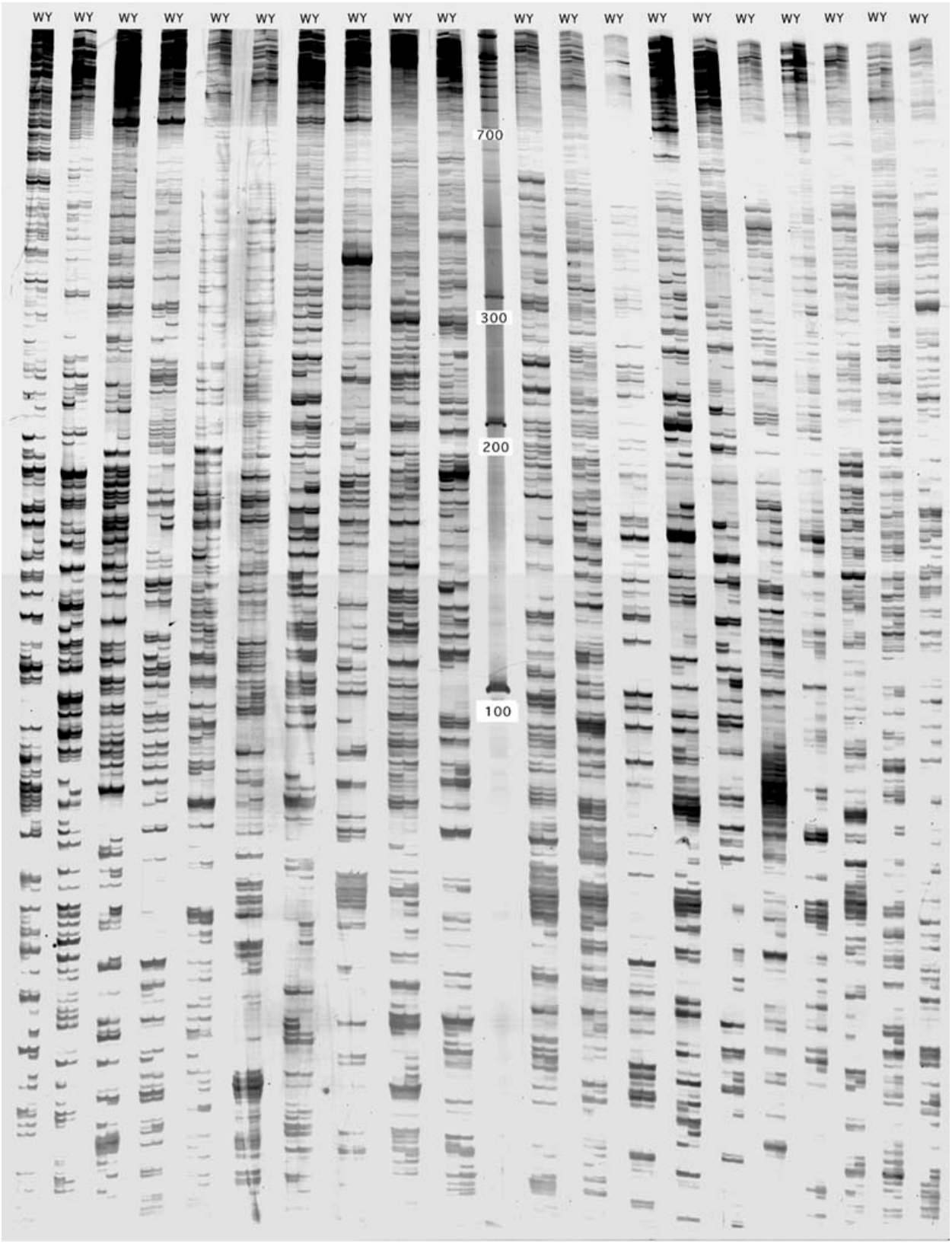


Figure 2. (Next page) AFLP polymorphism between FHB-resistant wheat Yiyuan 2 and FHB-susceptible wheat Wheaton, revealed by silver staining. Y: Yiyuan 2; W: Wheaton. Primer sets (from right to left): E ACA/ M-CTG, E-ACT/M-CAA, E-ACT/M-CAT, E-ACT/M-CAC, E-ACG/M-CAT, E-ACG/M-CTA, E-ACG/M-CAC, E-ACG/M-CTC, E-ACC/M-CAA, E-ACC/M-CAT, E-ACC/M-CTA, E-ACC/M-CTT, E-AGC/M-CAC, E-AGC/M-CAG, E-AGG/M-CAA, E-AGG/M-CAT, E-AGG/M-CAC, E-AGG/M-CAG, E-AGG/M-CTC, E-AGG/M-CTG; The numbers in the middle are the DNA standards (bp).

Figure 2.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BTH-INDUCED GENE EXPRESSION IN WHEAT SPIKES DOES NOT PROVIDE RESISTANCE TO SCAB

G-Y. Yu and G. J. Muehlbauer*

ABSTRACT

To identify control measures for wheat scab, we investigated the potential of using benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH). BTH induces a set of genes referred to as wheat chemical induction (WCI) genes and provides resistance to pathogens in several crops. Systemic acquired resistance (SAR) has been generally associated with BTH application. We sprayed a 1 mM BTH formulation on wheat plants several days prior to anthesis and examined gene expression and resistance to scab. We examined wheat spikes and flag leaves of BTH-treated wheat for expression of pathogenesis-related (PR) genes that are commonly associated with SAR, and the five WCI genes that are specifically induced by BTH in wheat leaves. We found that all five WCI genes were induced by BTH in both flag leaves and in spikes, however, none of the six PR genes tested were induced. In our disease evaluations of BTH-treated plants, we found that this treatment did not provide significant resistance to point or spray inoculations of *Fusarium graminearum*. These data indicate that BTH application and the induction of WCI gene expression does not provide resistance to scab.

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